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ABSTRACT

This syllabus begins with a list of program objectives and performance criteria for seven general topic areas related to the study of microorganisms and a list of 23 science processes. Following this information, concepts and understandings for subtopics within the general topic areas are listed as follows: (1) introduction; (2) the cell (basic structure, reproduction of plant/animal cells, cell and continuity of life, cellular biology); (3) the microscopic world (simple/compound microscopes, microscopic techniques, observation of pond water, bacteria, viruses); (4) techniques of isolating and culturing microbes (culturing bacteria, cultivation of viruses); (5) microbes and disease (difinition of disease, diseases in animals); (6) activities of non-pathogenic microbes (soil, sewage treatment, foods); and (7) control of undesirable microbes (sterilization, disinfection, preparation and storage of food, checks on product quality). Questions and activities corresponding to these concepts and understandings are keyed to a list of science activities presented in an appendix. These activites are also keyed to one or more of the processes listed at the beginning of the syllabus. Instructions for conducting the activities are also provided in the appendix. (JN)

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MICRO-ORGANISMS

LIVING SYSTEMS block **C**

SCIENCE SYLLABUS

FOR

MIDDLE AND JUNIOR HIGH SCHOOLS

BLOCK C

LIVING SYSTEMS: MICROORGANISMS

The University of the State of New York
THE STATE EDUCATION DEPARTMENT
Bureau of General Education Curriculum Development
Albany, New York 12234

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FOREWORD

This publication is the fourth product of the project to significantly update and reorder the Science Syllabus for Middle and Junior High Schools. The project will result in a group of instructional blocks encompassing the content and skills in the biological, physical, and earth sciences appropriate for those grade levels.

The understandings, questions, and learning activities in the publication have been enhanced by the inclusion and referencing of program objectives, performance criteria, and process skills which serve to focus student achievement. Thus, environmental considerations are infused throughout the content material and the importance of consumer and career implications in a science program is specifically acknowledged.

The objectives and performance criteria are provided in a general outline on pages 1 through 3 which conforms to the content subdivisions in the publication; the process skills are keyed to the learning activities; and the learning activities are keyed to the understandings and the questions.

The development of this syllabus was coordinated within the Bureau of General Education Curriculum Development by Barry W. Jamason, associate. Joan Rosner, (retired) Queens, District 30, revised major portions of the content material while reviewing the entire manuscript. Edward T. Lalor, Chief, Bureau of Science Education, and Thomas A. Boehm, associate in science education, reviewed all materials and made valuable suggestions for content improvement and accuracy. The cover design was created by Oscar A. Kaufman, Oaktree Films.

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PROGRAM OBJECTIVES AND PERFORMANCE CRITERIA

The program objectives and performance criteria below are provided as an aid in ordering the direction and synthesizing the outcomes of the instruction related to this block. The objectives and outcomes (performance) described below for this program permit latitude in the selection of content. It is implied that student knowledge, understanding, and skills accrued as a result of content selection and instruction will be demonstrable in the manner, and to the minimum extent, described.

Program Objectives	*Performance Criteria*
I. MICROORGANISMS	
The student will be able to cite the properties that characterize microorganisms as living.	Given an example of a living microorganism, the student will describe the properties that characterize it as living.
II. THE CELL	
The student will be able to describe the basic structure of the cell.	Given a cell model, the student will locate the name and its parts.
The student will be able to describe the functions of the cell parts and their role in the continuity of life.	Given a cell part, the student will define its function in the cell.
The student will be able to understand implications of recent developments in cellular biology as they affect society.	Given a situation involving genetic rearrangement, the student will be able to cite societal advantages and disadvantages of such activity.
III. THE MICROSCOPIC WORLD	
The student will be able to demonstrate the technique of operating a compound microscope and preparing a "wet mount" slide for observation.	Given a compound microscope, slide, and specimen source, the student will be able to demonstrate how the specimen would be mounted and observed under low and high power objectives of the microscope.
The student will be able to differentiate between the structural and functional properties of organisms found in pond water.	Given an appropriate organism, the student will be able to classify it as animal, plant, or protist.

Program Objectives	*Performance Criteria*
IV. TECHNIQUES OF ISOLATING AND CULTURING MICROBES	
The student will be able to describe basic techniques of culturing bacteria and viruses and the factors affecting infection, transmission, and defense against disease.	Given a situation involving culture preparation, the student will be able to describe proper technique.
V. MICROBES AND DISEASE	
The student will be able to define disease and differentiate between infectious and noninfectious diseases.	Given an appropriate disease name, the student will be able to classify it as infectious or noninfectious. Given a situation which describes an infectious disease, the student will be able to relate the ways that the disease infects and the defense methods used to halt its spread.
VI. ACTIVITIES OF NONPATHOGENIC MICROBES	
The student will be able to cite the importance of microbes to soils, sewage treatment, and some foods.	Given a situation which involves the functions of helpful microorganisms, the student will describe the role of these microorganisms.
VII. CONTROL OF UNDESIRABLE MICROBES	
The student will be able to describe the methods used to control undesirable microbes.	Given a method of microorganism control, the student will describe how the control operates to limit the growth of microorganisms.

Throughout this program the instructor should be cognizant of the importance of including several related objectives and performance criteria which represent the practical infusion of career and consumer considerations as they relate to science education:

The student will be able to relate both the positive and negative roles of microorganisms in the environment and to explain the necessity for selective control methods by man.	Given a case study which describes a disease prevention program which eliminated useful microorganisms or higher organisms as a side effect, the student will be able to describe possible alternatives to be examined.

Program Objectives	*Performance Criteria*
The student will be able to describe the career opportunities and the knowledge, skills, and attitudes helpful in those careers and occupations allied with the subject matter.	Given a career or occupational classification related to the subject material, the student will describe activities carried out in this work. Given an occupational role related to the subject material, the student will describe the training and aptitudes required to perform the role. Given an opportunity, the student will describe currently practiced work habits, skills, and knowledge that relate to careers implied in the subject material.
The student will be able to cite a range of possible occupational titles related to microbiology.	Given a career or occupation title, the student will be able to describe what would be expected of a prospective employee and to name organizations that would employ such a person.
The student will be able to demonstrate those consumer skills necessary for protection against disease.	Given a situation involving buying a food product, the student will relate the inspections to be made and any storage techniques necessary to control its spoilage.

PROCESSES

One of the major objectives in all science courses is the development of competence in processes used in science. The learning activities in this publication will assist students in attaining this objective. Each activity is keyed to one or more of the processes listed below. For example, activity (C-1) involves processes [1], [11], and [18]. In general, the processes are listed in order of predominance within the activity and appear to the right of the activity's title.

[1] Observing objects and phenomena

[2] Describing observations

[3] Selecting suitable measuring instruments

[4] Developing skills in using laboratory equipment

[5] Measuring objects and phenomena

[6] Performing laboratory techniques

[7] Estimating measurements within limits of accuracy

[8] Recognizing a problem

[9] Formulating a working hypothesis

[10] Designing appropriate tests of an hypothesis

[11] Designing suitable procedures for experimental tests

[12] Processing experimental data

[13] Organizing data to show a functional relationship

[14] Interpreting observations and data

[15] Interpolating and extrapolating

[16] Evaluating an hypothesis in the light of data obtained

[17] Formulating generalizations based on relationships

[18] Recognizing the need for a theoretical model

[19] Formulating a theoretical model to accommodate knowledge

[20] Designing relationships satisfied by a model

[21] Deducing an hypothesis from a theoretical model

[22] Interpreting and evaluating tests of a model

[23] Producing a revised, refined, or extended model

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
I Introduction	
Living things are characterized by certain properties which distinguish them from nonliving things.	*How do dead things differ from nonliving things?*
There is evidence which indicates that life was formed on Earth when energy derived from lightning, radioactive elements, and the sun's radiation broke apart simple compounds in the atmosphere (water, ammonia, methane, and other hydrocarbon gases) and recombined them to form complex organic molecules.	*Why is Earth the planet in our solar system apparently best suited to support life?*
	Based on our understanding of the continuity of life, what might be one reason that it is considered so important to protect endangered plant and animal species?
II The Cell	
Every living organism is made up of cells which carry on the same basic life functions as the organism itself.[1, 2]	*Diagram and label a generalized cell.*
A Basic structure	*Examine diverse types of plant and animal cells. What characteristics do they have in common?*[2]
1 cell membranes	
Cell membranes enclose the cytoplasm of the cell, and regulate passage of materials into and out of the cell.[3]	*How can a typical cell be depicted?* (C-1)
2 nucleus	*What are some of the principal materials moving into and out of cells through the membranes?*
The nucleus contains the chromogenetic material needed for duplication of the cell, and influences all cellular activity.	

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[1]There are two major types of cells: prokaryotes (pro-before; karyote - nucleus) and eukaryotes (eu - true). Bacteria and blue-green algae - both the earliest fossils and modern forms of these organisms - are prokaryotes. All larger and more complex cells in both plants and animals are eukaryotes. Basic parts of eukaryotic cells are discussed here.

[2]Consideration of the similarities in cells is emphasized here. It would be well to keep in mind that cells, in actuality, have a variety of different functions, such as movement, protection, and transport.

[3]Membranes are composed of lipids in which proteins are embedded. This is known as a mosaic model. Those proteins associated with the surface

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
a nuclear membrane	
The nuclear membrane encloses the nucleus, and controls the passage of materials between the nucleus and the surrounding cytoplasm.	*What roles do diffusion and osmosis play in the passage of materials through the cell?* (C-2)
b chromosomes	
Chromosomes, which are made up of DNA and protein, contain the information necessary for the growth, development, and maintenance of a cell. This information is replicated and passed on to each new cell when a cell reproduces. Each species seems to have a definite number of chromosomes.[4]	
(1) genes	
A gene is a specific area on a chromosome which determines a particular characteristic.	
(2) DNA	
DNA stands for a chemical compound called deoxyribonucleic acid. The Watson-Crick Model of a DNA molecule, which is a double-stranded helix shaped like a twisted ladder, shows how the molecule has both the stability to carry genetic information and to replicate itself.[5]	*Use a model of the DNA molecule to develop a better understanding of the molecule and its structure. Inexpensive tagboard kits are available for student use.*
(3) RNA	
RNA (ribonucleic acid) is similar chemically to DNA, but has a very different function. It is the transfer agent for the DNA, carrying the hereditary code from the DNA to the site of protein synthesis.[6]	*What does "breaking the genetic code" mean?* (C-3)
c nucleolus	
The nucleolus appears as a spherical body within the nucleus. It is the place in which ribosomal RNA is formed.	

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are called peripheral proteins. Those embedded within the lipid matrix are called integral proteins.

[4]Examples of chromosome numbers are: man - 46; fruit fly - 8; dog - 78; garden pea - 14; earthworm - 32; and frog - 26.

[5]The sides of the ladder are formed by molecules of sugar and phosphates, the rungs are formed from combinations of four different nitrogenous bases (adenine-A; thymine-T; guanine-G; and cytosine-C). Guanine is always paired with cytosine; adenine with thymine. Weak hydrogen bonds hold these bases together. When a chromosome replicates itself, these

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
3 cytoplasm	
The cytoplasm is that portion of the cell substance outside the nucleus and within the cell membrane. It contains functioning structures called organelles.	*Describe the area of the cell that is made up of cytoplasm.* *What are organelles?*
a mitochondria	
Mitochondria are organelles which are the sites of the oxidation of food molecules and of energy transfer. They are the main centers for the formation of ATP which is the principal carrier of energy in the living organism.	*Why is ATP such an important substance in the body's chemistry?*[7]
b endoplasmic reticulum	
This network of tube-like membranes divides the cell into compartments which provide a sequential, assembly-line type organization in which the enzymes carrying out the cell's many varied activities can be separated as they function.	*What are the known functions of the endoplasmic reticulum? (intracellular transport; lipid synthesis; lipid storage; and, drug metabolism)*
c golgi bodies	
The golgi complex takes part in the polymerization and secretion of carbohydrates (cellulose in plants). They are also closely associated with the synthesis and secretion of glycoproteins (mucus).	
d ribosomes	
Ribosomes are organelles located along the endoplasmic reticulum which serve as sites for the synthesis of enzymes and other proteins from amino acids.	
e chloroplasts	
Chloroplasts are organelles that contain the green plant pigment, chlorophyll. Photosynthesis takes place in chloroplasts.	

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bonds break and a separation occurs. Then each half develops a complementary area and forms a new chromosome exactly like the original.

[6]Structurally, RNA differs from DNA in three ways. First, instead of the base thyamine, RNA has the closely related uracil. Second, the sugar molecules (ribose) contain one more oxygen atom than the sugar molecules (deoxyribose) in DNA (deoxyribonucleic acid). Third, the RNA is usually single-stranded, and most RNA does not have a regular helical structure. There are three types of RNA. Basically, their

CONCEPTS AND UNDERSTANDINGS

QUESTIONS AND ACTIVITIES

B Reproduction of plant and animal cells

1 mitosis

Mitosis is a process which results in two nuclei which are identical to the parent nucleus. For descriptive purposes, the process of mitosis is divided into five phases or stages. The chromosomes replicate and make exact copies of themselves (interphase). The chromatin material shortens and thickens. In animal cells, asters form at opposite sides of the nucleus (prophase). The replicated chromosomes line up equatorially in the cell. Spindle fibers may be evident (metaphase). The chromosomes are drawn apart (anaphase). New nuclear membranes are formed and two daughter nuclei result which have identical genetic material (telophase).

Make simple diagrams of the five phases of mitosis. What takes place in the nucleus of a cell during each of the four phases?

2 cytoplasmic division

Division of the cytoplasm usually accompanies mitosis. The result is two daughter cells with identical genetic material.[8]

3 mitotic cell division

Mitotic cell division is a process which includes nuclear division and cytoplasmic division. This process results in two cells which are identical to the parent cell.

Cell production is basic to the continuity of life. For example, a fertilized egg divides and the new cells divide until an adult organism is formed.[9]

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function relates to combining amino acids in the proper sequence during the process of protein synthesis.

[7]ATP (adenosine triphosphate) is the principal carrier of energy in the living organism. When food molecules are broken down with the resultant release of energy, some of this energy is caught and stored as ATP, then released to supply the energy for most bodily activities.

[8]Cytoplasmic division usually begins during the telophase. The cleavage always begins at the midline of the spindle, and the cell is usually divided in half. Cytokinesis differs somewhat in plant and animal cells.

[9]Cell division goes on at different rates according to the circumstances. Emergency measures, such as skin injuries or loss of blood, cause

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
C The cell and continuity of life	
1 meiosis	
Meiosis is a process of nuclear division which takes place in two successive stages, and which results in the formation of gametes (or sex cells) each of which has half the number of the original chromosomes.	*What is the difference between meiosis and mitosis which prevents the fertilized egg from having twice as many chromosomes as are found in the somatic (body) cells of the parent organism?*
Crossing over of genetic material, which occurs during meiosis, leads to increased possibility of variation in the next generation.	
2 mutations	
A mutation involves a sudden change in the genetic material of a cell. The alteration occurs either in the chemical makeup of a gene or in the structure or number of chromosomes in the cell and will therefore be reproducible.[10]	*What are some of the factors which cause mutations?*
Mutations may increase or decrease the ability of a cell to adapt to its environment. Some mutations are even lethal to the organism.	*How have mutations in bacteria made some antibiotics less effective than they were originally?*
Artificial radiation dramatically increases the rate of mutations beyond the normal incidence.	*What are some of the hazardous environmental pollutants which are thought to cause birth defects?*
3 genotypes and phenotypes	
Genotype refers to the genetic makeup, or genetic potential, of an organism. Its phenotype is determined by the interaction between the genotype and the internal and external environment.	*Based on a knowledge of heredity, why is it surprising to find that many teenagers throughout the world have been taller than their parents during the past few decades? How might this be explained?* (C-6)
D Cellular biology	
Cellular biology is a rapidly evolving field. Every discovery leads to many new questions. It is important that students at all school	*What evidence supports the idea that some forms of cancer are caused by viruses?*

the body to increase its rate of production. The process of mitosis is similar for plant and animal cells.

[10] Mutations may account for hornless Hereford cattle, insects resistant to DDT, and microorganisms resistant to penicillin.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
levels understand the dynamic nature of this discipline, and that they are aware of new breakthroughs as they occur.	*What is cloning? What could be the advantages of artificial DNA recombination? The disadvantages? What role are citizens in some communities playing in decisionmaking about genetic engineering research underway at nearby universities?*
III The Microscopic World	
A microbe is an organism which is too small to be seen with the naked eye. The greatest number and variety of all living things fall into this category. There is a considerable variation in size — the largest are several thousand times the size of the smallest.	
A Simple and compound microscopes	*How can a simple "water drop" microscope be constructed?* (C-5)
	Have students make sketches of a basic lens system.
	Sketch the parts of a compound microscope and explain their functions.[11]
B Microscopic techniques	*What are the basic rules for using the microscope?*[12] (C-6)
1 slide preparation and simple staining	
Simple staining is useful to outline the boundaries of the cells and the organelles within them. Staining makes observation easier.[13]	*How is a stain applied to a slide?* (C-7)

[11]Emphasis in this unit will be on the use of the compound microscope. It is recommended that there be at least one microscope for every two pupils, since this entire block is focused upon its use.

[12]Rules for use of the microscope:

Always carry the microscope with two hands, one on the arm and one under the base. Never support it by the monocular tube.
Never turn the adjustment knobs forward while looking through the eyepiece.
Clean the lenses with lens paper only.
Be sure adequate light is available. Do not use direct sunlight.
Never use the coarse adjustment when viewing a slide under high power.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
2 simple measuring	
Microns (μ) and millimicrons (mμ) are used to measure microscopic organisms.[14]	*If a human red blood cell (7,500 microns) is drawn with a 20-cm diameter, how should an influenza virus (115 millimicrons) and a polio virus (25 millimicrons) be drawn?* (C-8)
C Observing pond water	
1 "green" organisms	
Most living things which are green can be classified as plants or protists. They are capable of carrying on photosynthesis. Green organisms are the first step in food chains, converting the sun's energy into energy readily available to other living things.	
Blue-green algae are the simplest green organisms and probably resemble some of the earlier forms of life on Earth.[15]	*What causes "red tide," and what effect does this phenomenon have?*
Unchecked growth of algae in a pond or lake is called eutrophication. It occurs naturally, and normally results in the "death" of a body of water after thousands of years. Excessive amounts of nitrates and phosphates in the water speed up the process of eutrophication.[16]	*What is eutrophication?* *What effect does the dumping of sewage and agricultural waste into a lake have on its ecology?* *What efforts have been made to clean up our polluted waters, and how successful have they been?*

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[13] For introducing staining techniques, methylene blue, gentian violet, or iodine applied lightly are useful.

[14] 1 millimicron = .001 micron (μ)
1 micron = .001 millimeter (mm)

ameba = more than 100 microns (μ)
bacteria = .3μ to 40μ
rickettsiae = .2μ to .3μ
PPLO = .1μ to .25μ
viruses = .08μ to .3μ

[15] Blue-green algae are prokaryotes. They are unicellular, and they do not have complex chromosomes, endoplasmic reticula or mitochondria. Most prokaryotes have rigid, noncellulose (polysaccharide plus amino acid) cell walls, as distinguished from the cellulose cell walls of most plants. In one system of classification, eukaryotes are classified into six phyla, three of which are unicellular and are

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES

2 nongreen organisms

Nongreen organisms observable in pond water are either animals or protists. If there is a cell wall surrounding the plasma membrane, the organism belongs to the group of protists known as fungi.

a protozoa

Protozoa are unicellular protists.

What similarities can be noted among the microscopic organisms seen in a drop of pond water?

What are some of the ways they differ from each other? (C-9)

b fungi

Fungi are protists which have cell walls but lack chlorophyll, and consequently must obtain energy from other living things. Parasites get nutrients from living hosts; saprophytes from nonliving sources.

What kinds of organisms are included in the group known as fungi?

What distinguishes fungi from other organisms?

Fungi secrete enzymes that penetrate nutrient materials and digest it externally and, as a result, the food can be absorbed into the cell.

Most fungi are aerobes (they need oxygen), but their oxygen requirements may be quite low. Some fungi are capable of surviving in anaerobic (without O_2) conditions but will grow faster in O_2.

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considered protists. The most familiar examples of these three phyla are: euglena, diatoms, and "fire" algae (dinoflagellates). Euglena have characteristics of protozoa, and were classified as animals until recently. They have a contractile vacuole, lack a cell wall, and are capable of locomotion. Diatoms, which are the principal organisms amongst phyoplankton, have rigid cell walls which contain no cellulose. Dinoflagellates are often red, and are responsible for the "red tides" which have caused the death of thousands of fish.

[16]When algae floating on the surface of a body of water are "overfed" by phosphorus and nitrate wastes

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
D Bacteria	
Bacteria are the simplest, the oldest, and the most abundant group of organisms in the world. They are one-celled organisms which have a tough cell wall. Most bacteria are nonphotosynthetic, nonmotile, diversified, and well-equipped for survival.[17]	*What are some of the reasons bacteria are so abundant?*
1 reproduction Bacteria reproduce by a process called binary fission.	*What is binary fission?* *If it takes 24 hours to half fill a flask with bacteria which divide every 15 minutes, how long would it take to fill the flask?*
2 true bacteria (Eubacteria)	*Identify the chief components of a bacterial cell.*
a shapes There are three basic cell shapes among true bacteria: rod-shaped bacteria are called bacilli; round or spherical bacteria are called cocci; and spiral-shaped are called spirilli.	*How are true bacteria identified and classified?* *Where are microbes found?* (C-10)
b arrangements Certain bacteria grow in characteristic groupings: paired, spherical bacteria are called diplococci; chains are called streptococci; and clusters are staphylococci.[18]	*What are some common diseases of humans which are caused by true bacteria?*
3 other bacteria Three other classes of bacteria are: Mxyobacteria ("slime"); Spirochetes, which	

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dumped in the water, they grow geometrically, forming a thick layer over the water. As they carry on their photosynthesis, they absorb the sunlight falling on the water, preventing plants at lower levels of the water from photosynthesizing. The oxygen supply at these deeper layers is rapidly used up as plants and animals die and decompose, and no new oxygen is added due to the lack of photosynthesis. Fish at the bottom die because the oxygen supply is depleted, and their decomposition further exhausts the available oxygen. The ecology is completely altered, and the water becomes a mass of muck and green slime; a dead environment.

move with a whirling motion; and Rickettsia, which cause Rocky Mountain Spotted Fever and Typhus.

E Viruses

1 living or nonliving?

There is much still to be learned about viruses. They do not fit into any of the standard categories used for other organisms, and there is widespread debate as to whether they are living organisms. Viruses do reproduce, but only in the presence of other living cells.[19]

2 structure

Viruses are composed of an inner core of nucleic acid, either RNA or DNA, and an outer coat of protein.[20, 21]

What instrument is used to observe viruses?

3 viruses and disease

Viruses are responsible for such diseases as smallpox, chicken pox, measles, colds, influenza, yellow fever, polio, cold sores and shingles (the latter two caused by Herpes simplex).

There is increasing evidence to indicate that some forms of cancer, and multiple sclerosis, are caused by viruses.

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[17] They can double in size in 20 to 30 minutes under optimum conditions, and can live in many environments too harsh for other organisms. Several species can form thick-walled spores which enable them to retain viability for many years. Some bacteria can live with or without oxygen. Some can live only without oxygen. Three forms of bacteria are photosynthetic, and others are chemosynthetic (deriving energy from the oxidation of inorganic compounds).

[18] A diplococcus organism causes pneumonia. Streptococcus organisms may cause throat infections and blood poisoning. Staphylococcus organisms cause boils, abscesses, and pimples. Identification of bacteria may be made by differential staining methods such as the Gram stain technique.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
IV Techniques of Isolating and Culturing Microbes	
In order to study microbes more closely, we must be able to separate the species.	
A Culturing bacteria	
1 bacteriological methods	*To what extent were Koch's postulates employed in research related to "Legionnaire's disease?"*[22]
Bacteriological methods may be used to grow and isolate yeasts, molds, and bacteria.	
a maintaining sterility	
All equipment must be cleaned and sterilized before use, and the sterile techniques must be observed at all times.[23]	*What effect does chlorine have on microbes?* (C-11)
b culture media	
A culture medium is a nutrient preparation which provides the food needed by the microbes.[24]	*What are some typical culture media, and how are they prepared?* (C-12)
c general rules	*What are the optimum conditions for bacterial growth?* (C-13)
Cultures of bacteria should be treated with care because of the possible heavy concentration of pathogenic organisms.[25]	*What are the pros and cons of the use of disposable plastic items in laboratories, hospitals, and school lunchrooms?*[26]
2 colonies	
A colony is a group of microorganisms growing together.[27]	*In what ways do the colonies formed by various species differ from each other?* (C-14)
Each colony usually indicates a single bacterium placed on the culture medium.	

[19]Some researchers believe that viruses are cellular fragments that live partially independent of the living organisms from which they came. S. E. Luria, who received the Nobel prize for work with bacteriophage, called viruses "bits of heredity looking for a chromosome." When viruses reproduce, DNA or RNA is released within a cell. Using parts of the host cell, the DNA or RNA reproduce many new viruses, which enter other cells and repeat the process.

[20]The electron microscope will magnify up to 25,000 diameters, and an image from the electron microscope may be enlarged to 300,000 diameters.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
Bacteria form definite colonies or masses which will have distinctive characteristics of size, texture, shape and color.	*How can microscopic examination be used to establish the identity of the bacterium which caused formation of a colony?* (C-15) *How can a pure culture be obtained?* (C-16)
B Cultivation of viruses Viruses cannot be grown in an artificial, nonliving media. They will only grow inside living cells. Fertilized eggs are used to culture viruses. A virus is injected into the egg, and it invades the cells of the embryo and reproduces viruses.	*How are vaccines for such viral diseases as yellow fever and influenza made?*
V Microbes and Disease	
A Definition of disease Disease may be defined as a condition in which the normal body functions of an organism are disturbed or impaired. Diseases may be classified in two basic groups - infectious and noninfectious.[28]	
1 infectious diseases Many infectious diseases are caused by microscopic organisms, and are usually spread from one organism to another.	
2 noninfectious diseases Noninfectious diseases are caused by such factors as deficiencies in diet, malfunctioning endocrine glands, allergies	*Why might it be more correct to speak of cancer as "noncontagious" rather than noninfectious?*

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[21] DNA or RNA is released within a cell. It then takes over and, using the parts of the cell, reproduces a multitude of new viruses which are then released to enter other cells and continue the process.

[22] Robert Koch, in attempting to establish with certainty the causative agent for a particular disease, developed what are known as "Koch's postulates":

(1) The organism suspected of causing the disease must be present in every infected individual.
(2) The suspected pathogen must be isolated and grown in a pure culture.
(3) Some of the pure culture must

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
to foreign substances, malfunctioning organs, and diseases caused by chemicals introduced into the environment.[29]	*How has the rapidly growing highway system helped to encourage growth of ragweed—a principal cause of hayfever?*
B Diseases in animals	
Disease-producing organisms are called pathogens.	
1 infection	
Infection is the entrance, growth, and multiplication of a pathogen in the body of a host which results in disease.[30]	*How much validity is there in the old admonition, "Don't go out in this weather or you'll catch your death of cold?"*
Infection depends on several factors: resistance of the host, virulence and numbers of the pathogen, and the route of entry of the pathogen.	*What effect might the chemical preservatives in some of our over-refined foods have on our digestive system if they destroy some of the harmless bacteria in the intestines?*
2 transmission of pathogens	
A pathogen must be transferable from one host to another in order to ensure its own survival.	*How are pathogenic bacteria spread?* (C-17)
Since most microbes have little or no self-locomotion, they must be transmitted by one or more agents which are called vectors.[31]	*Why is there still controversy in some parts of the world about whether or not DDT should be banned?*
	What does a high coliform bacteria count in water indicate about the amount of sewage that has been discharged into that body of water?

be injected into healthy organisms and cause the disease.

(4) The pathogen may again be removed from diseased organisms and regrown and compared with the original pure laboratory culture.

[23]Sterile conditions may be obtained by using incinerating heat (an autoclave or a pressure cooker), or by the use of chemicals such as sodium hypochlorite (household bleach).

[24]Pupils should know that different types of microbes have different food preferences. Some will grow best in a nutrient with a base of beef broth, blood, or vegetable matter.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
3 body defenses	
Although microbes of many kinds constantly inhabit the skin and body cavities, the natural defenses of the body manage to keep humans remarkably free of disease.	*How can the normal bacteria of the skin be cultured and observed?* (C-18)
a structural defenses	
Parts of the body form physical barriers against microbial invasion.	*How do air pollutants affect the cilia and mucous membranes in the nasal and respiratory passages?*
. The skin prevents or limits the entrance of microbes.	
. Mucus secretions in the respiratory, alimentary, and urogenital tracts trap invading organisms. Cilia lining the nasal passages and respiratory tract clean the air which is being inhaled.	
. Hydrochloric acid in the stomach inhibits microbes which enter this portion of the body.	
. Tears wash away microbes which enter the eyes.	
b phagocytic leucocytes	
Phagocytic leucocytes are white blood cells which concentrate rapidly in an inflamed area, and dispose of foreign organisms by surrounding and ingesting them much as an ameba engulfs its food.[32]	*How can phagocytes be observed?* (C-19)

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[25]The following rules should be observed:

(1) Treat all microbes as though they are pathogenic (disease-causing) organisms, even though most microbes are not normally disease-causing.
(2) Do not eat or drink in the laboratory. Keep fingers, pencils, etc., out of the mouth.
(3) Wash the hands thoroughly with soap and water before and after doing any laboratory work.
(4) Sterilize all needles and loops in an open flame before and after using them.
(5) Sterilize all cultures before discarding them. Wash only petri dishes or tubes which have been sterilized first.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
c antibodies and antigens	
Antibodies are protein substances which defend the body against foreign proteins known as antigens. They do this by: (1) neutralizing toxins; (2) dissolving microbes; or (3) agglutinating cells so they may be more easily engulfed by leucocytes. Antibodies are produced by lymph nodes, spleen, and thymus gland.	*What are some of the ways antibodies defend the body against foreign protein substances?*
4 immunity	
Immunity is an ability to resist a disease.	*Why was there so much controversy related to Swine Flu vaccination during the winter of 1976-77?*
a active acquired immunity	*What are some of the most important diseases which have been controlled by vaccination during the past 100 years?*
If the antibodies are developed by the body itself, either in response to an actual case of the disease or a vaccination of dead or weakened organisms, the immunity developed is called an active acquired immunity.[33]	*What role has immunization to deadly diseases played in helping create the over-population/famine syndrome currently seen in some developing countries?*
b passive acquired immunity	*Students should be encouraged to read books such as Paul DeKruif's Microbe Hunters and Sinclair Lewis' Arrowsmith.*
If the antibodies are developed in another organism and are then injected into the organism which is to be protected, the immunity is temporary and is called passive acquired immunity.[34]	

Plastic disposable plates should be incinerated and not reused. Much care must be taken to insure the destruction of the live cultures produced in the laboratory. They should be autoclaved before they are disposed of.

(6) In case a culture is spilled, notify the teacher immediately and disinfect the hands and area involved.

(7) Flushing with a strong disinfectant is not acceptable. Laboratory manuals now consider this method inadequate for the destruction of the microbes.

[26]From a sanitary point of view, the use of disposable plastics in

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
c antibiotics	
Chemical substances which are produced from living things, especially fungi, and which stop the growth of germs are called antibiotics. Penicillin, streptomycin, Chloromycetin, and Terramycin are among the best known examples of these "wonder" drugs."[35]	*What protective reaction have bacteria shown to most of the "wonder drugs?"* *How can we test the effectiveness of antibiotics?* (C-20)
d natural immunity	
Natural immunity is an inborn, inherited resistance to a disease.	*Give examples of natural immunity.*
5 effects of infection on a host	
Effects on the body are specific for each pathogen.	
a destruction of tissue	
The tuberculin bacillus destroys tissue. Tuberculosis may occur in many parts of the body, such as lungs, bones, or glands.	*How many of the areas of the country formerly used as treatment centers for tuberculosis still have air clean enough for that purpose?*
b production of toxins	
Toxins are poisons produced by pathogens.	*What is botulism?*
In rabies, the viruses produce toxins which adversely affect the central nervous system.	
In tetanus, toxins from the bacillus severely affect the central nervous system.	

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laboratories, hospitals, and school and public eating places is indisputably a milestone. However, from an environmental viewpoint, the use of these items creates several very severe problems. The most alarming of these is the growing amount of solid waste generated in our "throwaway society." Many large cities are fast running out of places to dump their solid waste. Another, less obvious, problem is that of waste of important hydrocarbons which are basic ingredients of plastics, and which represent oil products perhaps better used in providing fuels to meet the world's energy needs. Disposable plastics sometimes present a third environmental problem when, in the process of getting rid of them, we burn them and add to our air pollution problems.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
c interference with normal functioning	
Some animal parasites cause serious diseases in animal hosts.[36]	
C Plant diseases	
Plant diseases pose a threat to man's survival by destroying food plants or by limiting their production.	*How is it possible to determine virus infections in plants?* (C-21)
1 molds	
Molds are found in dust and decomposing organic matter. Certain types destroy ropes and timbers that are exposed to water by decomposing cellulose.	*What is monoculture and why is it ecologically unsound?*
2 yeasts	
Yeasts are found in such places as dust, soil, and on grapes. Diseases caused by a yeast organism are Dutch Elm Disease, Chestnut Blight, and ergot disease of rye.	
3 virus	
Plant viruses are very specific. Some affect only the leaves, or the stem, or some other part of the plant.[37]	
Much of the earliest research work on viruses was done with the "tobacco mosaic" virus.[38]	

[27]One bacterium may produce 16 million bacteria in 24 hours and form a visible spot called a colony. Large, sprawling filaments on a culture plate are molds. They may vary in color but are mostly cottony, fibrous-like growths.

[28]Some pupils may view all diseases as infectious. Some will have no idea at all about the cause of disease. Emphasis in this unit will be on infectious diseases, but some examples of noninfectious diseases should be discussed. Cancer is usually categorized as noninfectious—although there is strong evidence that several forms of cancer are caused by viruses—because it is not contagious as most infectious diseases are.

[29]Statistics indicate that humanmade health hazards have supplanted

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES

VI Activities of Nonpathogenic Microbes

There are untold numbers of microorganisms in existence, but only about 5 percent of them seem to be pathogenic. The rest are vital to the ecological balance of all life, but often go unnoticed and unappreciated because their niche is not brought dramatically to our attention. Without them, decay would not occur, the intestines would not function properly, and many foods would be tasteless.

A Soil

Soil microbes include bacteria, molds, algae, protozoa, and some yeasts.

How can pupils demonstrate the presence of microbes in soil? (C-22)

It is predicted that approximately 1 billion viable cells will be found in 1 gm. of fertile, moist soil.[39]

Soil, and the microorganisms in soil, play a vital role in the life cycles of all organisms, but people—through their ignorance of the importance of soil—do many things to disturb these ecological relationships.[40]

What must be emphasized about the ecological importance of soil?

Soil pH must be regulated to permit optimum growth of crop plants and it is important to soil microorganisms.

1 biochemical cycles

The oxygen—carbon dioxide cycle, the nitrogen cycle, and the sulfur cycle are all dependent upon the activities of microorganisms as they simplify organic material by processes of decay.[41]

What would be the consequence if all nitrogen-fixing bacteria were killed by a pesticide?

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infectious diseases as causes of death. Several factors are responsible for this trend which has been developing since the turn of the century. An important one is that improved sanitation, development of immunization techniques, and use of antibiotics have almost completely controlled many infectious diseases once dreaded as epidemic killers. More people now live to grow old and to be subjected to diseases associated with aging. Concurrently, the number of pollutants poured into the environment has increased. Pesticides, harmful food preservatives, chemicals associated with plastics, automobile emissions such as carbon monoxide and other air pollutants which affect the lungs—are just a few of the

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
	Why do good farming practices dictate rotation of crops and the planting of a legume in alternate years?
2 decay	
Decay is an all-important process involving the conversion of complex organic materials into simpler organic and inorganic substances.	*How has the solid waste disposal problem been aggravated by the current extensive use of nonbiodegradable substances such as plastics?*
All living things are ultimately degraded into usable materials for the growth of other living things. The decayed materials may serve as foods or fertilizers for other living things.	*What is composting, and why is it considered an environmentally-sound use of some domestic and agricultural waste?*
Matter and energy are never lost, but they do change from one form to another.	
B Sewage treatment	
Sewage is 99 percent water, but the other 1 percent is made up of such things as urea, proteins, carbohydrates, fats, soaps, and detergents. The purpose of sewage treatment is to convert the organic materials to inorganic materials.	*How do primary, secondary and tertiary sewage treatment plants differ?*
Treatment of sewage is an attempt to accelerate nature's process of decay.	*How adequate are the majority of sewage treatment plants in the United States? In New York State? In your city?*
1 anaerobic bacteria	
After the large particles in sewage have been removed by settling or filtering, anaerobic bacteria begin their work of digesting the	*Plan a field trip to a local river or stream to observe the effects of pollution.*

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concomitants of our modern life-style which are putting noninfectious diseases out in front as causes of serious illness and death.

[30]The presence of a pathogen in an organism does not necessarily mean that the organism will become diseased. Many microbes live in nasal cavities, throat, intestines, etc., and DO NOT cause disease. The proper set of conditions must be established before a disease can take hold.

[31]Vectors include insects, infected people, contaminated objects, food, water, dust, and air.

[32]The characteristic features of certain infections - fever, pain, redness, swelling, and pus formation - are part of the normal

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
waste. Methane gas is given off in the process, and may be recovered and burned as a fuel.	*Plan a visit to a sewage treatment plant.* *How will improved sewage facilities lead to an improved quality of life for us?*[42] *What roles do anaerobic bacteria play in sewage treatment? Aerobic bacteria?*
2 aerobic bacteria	
After anaerobic bacteria have decomposed the waste material, it is aerated with compressed air. Aerobic bacteria oxidize this material, releasing energy and producing sulfates, phosphates, nitrates carbon dioxide and water (all inorganic compounds).	*What attempts have been made to use the methane gas given off in sewage treatment as an alternate source of energy?*
C Foods	
Many foods, such as breads, cheeses, and wines obtain their characteristic flavors from cultures of microorganisms.	*How are foods partially made by the action of microorganisms?* (C-23)
1 yeasts	
Yeasts are used to ferment sugars to produce beverage alcohol under anaerobic conditions. Wines are made from fermented fruit juices and beers from fermented grains. The baking process uses yeasts to produce CO_2 which expands when heated to make bread rise.	*Observe the action of yeast.* (C-24)

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body reaction. During this process new tissue cells are forming in and around the area attempting to limit the site of the infection and to replace dead tissue with new tissue.

The location of the swollen lymph nodes may help to determine the site of infection. Special structures in the liver, spleen, and lungs filter out foreign particles.

"Pus" is composed of dead and living leucocytes, dead and living bacteria, tissue debris, and blood fluids.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
2 molds	
Molds are used in the production of various cheeses. Roquefort, Gorgonzola, and blue cheeses are produced by inoculating specific molds into the cheese curd.[43]	*Have pupils grow molds on substrates, such as potato, orange peel, bread, etc.* (C-25)
3 bacteria	
Silage, a cattle food, is prepared by placing finely chopped plants in silos to ferment.	
Many foods, including sauerkraut, yogurt, buttermilk, acidophilus, cottage cheese, and butter are prepared with the assistance of bacteria.	*Describe some bacterial actions on food.* (C-26) *Grow a culture of one or more of these products, and observe the organisms under the microscope.* (C-27)
VII Control of Undesirable Microbes	
To prevent infection, contamination, and spoilage, microbes are controlled by removing or killing them, or by inhibiting their growth.	
A Sterilization	
Sterilization is the process by which all living organisms (plant or animal) are destroyed. Sterilization is vital to the microbiologist.	*How can pupils show the effect of heat on bacteria?* (C-28)
Aseptic conditions are conditions in which complete sterility is maintained.	*How are sterile conditions maintained in the laboratory? What are the advantages and disadvantages of each method?*

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[33]Some diseases to which humans can acquire an immunity are measles, mumps, whooping cough, smallpox, and poliomyelitis. Edward Jenner's discovery that milkmaids never contracted smallpox led to the development of a smallpox vaccine prepared from the cowpox on the cow's udders. Antibodies may persist in the bloodstream for weeks, months, or even years, but there is a gradual decline in their numbers - thus the importance of booster shots for polio, tetanus, and many other diseases.

[34]Diphtheria antitoxin is developed in horses, and, when injected into humans, produces a passive acquired immunity to the disease. Temporary immunity is usually used to combat or protect against infection which is already present or imminent.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
B Disinfection	
Disinfection is the destruction of all pathogenic organisms. In some cases, sterilization may result but not always.	*How can pupils test the effectiveness of various chemicals in controlling bacterial growth?* (C-29)
Any agent that kills microorganisms is called a germicide. Bactericidal, fungicidal, and virucidal agents are specific types of germicides.	*How can pupils determine the best method of washing dishes?* (C-30)
	How influential are advertising campaigns in "selling" various brands of detergents?
	How have some detergents been harmful to the environment?
	What steps have been taken to correct the water pollution problems caused by the use of high-phosphate dishwasher detergent?
C Preparation and storage of food	
1 pasteurization	
The process of pasteurization kills most pathogenic organisms. Many products are pasteurized, such as milk and milk products, beer, wines, dates, and fruit juices.	*How can we determine the extent of bacterial life which remains in milk after pasteurization?*
The United States Public Health Service set 25,000 bacteria per ml. as the maximum number allowed in Grade A pasteurized milk. Raw milk may contain eight or more times this amount. Sour milk contains as many as 500 million living bacteria per ml.[44]	

[35]From the time of earliest microbiological studies, platings of fertile soil showed zones of inhibition around certain organisms. The substances producing these zones were considered nuisance contaminants until Alexander Fleming, in the late 1920's, realized the importance of this action. He isolated and named penicillin which is produced by a mold. Most antibiotics now in medical use are produced by molds or bacteria. Antibiotic substances may be broad-spectrum or specific. Pupils may be familiar with the inhibition assay test which some physicians use routinely to determine what medicine will do the most effective job of destroying the particular microbe responsible for causing an infection.

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
2 canning	
Home canning must be done carefully. Foods should be handled with high standards of cleanliness.	*Is the trend in the United States toward or away from home canning and preservation of food? Explain.*
Commercial canning includes sterilization of the product after it has been sealed in the can. This is achieved by a pressure of 15 lbs. per sq. in., a temperature of 121°C and a time of 20 to 30 minutes. This is sufficient to kill even highly resistant spores.[45]	*What recent examples have there been of botulism caused by commercially canned foods?*
Generally, acid foods will be less likely to spoil because the acidic environment is not conducive to optimum growth.	
3 drying	
Removal of the moisture which is necessary for microbial growth will inhibit the growth but not destroy the organisms. Thus, these foods must be kept dry. Moisture will cause immediate resumption of growth.	*What are some examples of foods preserved by drying?* *What foods do backpackers carry on their hiking trips?*
4 salt curing, pickling, sugaring, and smoking	
Curing with salt involves the destruction of microorganisms by making the salt environment too high for microbial life.	*Which of the foods in our normal diet are preserved by smoking? Pickling? Salting? Sugaring?*
Pickling is a means of preserving by making the acidity too high for microbial growth.	
Sugaring lowers the amount of available moisture for microbial growth.	

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[36]The malarial protozoan attacks and destroys red blood cells. Filarial worms block off lymph nodes and cause a condition known as elephantiasis. Other parasites, such as tapeworms, attach themselves to the walls of the intestines and ingest the digested food before the host is able to absorb it.

[37]Some very popular multicolored tulips originally developed as a result of viral infections.

[38]In 1892, a Russian botanist, Iwanowski, discovered that the sap from a diseased plant still caused the disease even after it was filtered through clay, paper, asbestos and porcelain. This

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
Smoking involves the preservative functions of products of wood burning, such as cresols, formic, acetic and other organic acids, alcohol, and formaldehyde.	
5 ultraviolet radiation	
Sunlight has been used as a means of destroying microorganisms. The ultraviolet rays are the most effective radiations present. They are even more capable of killing many spore-forming bacteria which are not destroyed by boiling.	*How can pupils observe the lethal effect which ultraviolet radiation has on microbes?* (C-31)
The limiting factor involved is the penetrating power of these rays. Presently, polluting factors associated with densely populated urban areas stop sun rays.[46]	*How do crowded urban conditions affect the amount of ultraviolet radiation which is able to reach and destroy microorganisms?*
	How polluted is the air in your community? (C-32)
6 refrigeration	
Prompt lowering of food temperature to the temperature in a refrigerator (about 4°C) inhibits the growth of microorganisms. It does not destroy them.	*What is the effect of refrigeration on milk bacteria?* (C-33)
Bacteria that cause milk to sour divide every hour at 21°C, every 2 hours at 16°C, and every 30 hours at 4°C.[47]	
7 freezing	
Freezing is a method of preserving things—even microorganisms. Freezing does not destroy them but does inhibit their growth.	*What demands do refrigeration and home-freezing make on our dwindling energy supply? What can the consumer and home-maker do to minimize these demands?*
Frozen foods should never be refrozen because the freezing process weakens the natural resistance of the fresh product.	

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same virus (TMV) has provided a good example of the structural use of proteins. Because of its simplicity, TMV has been considered a model system for studying biological self-assemblies.

[39]A tremendous competition for survival goes on continuously in soil. Protozoa consume bacteria. As the food supply for one type of microbe is used up, another type takes over. The type of microbe predominating fluctuates constantly in this never-ending process.

[40]People use tremendous quantities of fungicides, insecticides, and herbicides without considering that helpful decomposers as well as pests will be killed by their action. In the same way, they poison small

CONCEPTS AND UNDERSTANDINGS	QUESTIONS AND ACTIVITIES
D Checks on product quality	
In order to produce and sell safe products, many food industries maintain their own laboratories to test their finished products. Federal, State, and county health departments periodically check to see that certain standards, established by law to protect the public, are maintained.	*How effective is the U.S. Food and Drug Administration in maintaining high standards for commercially prepared foods?*
Where the count is high enough, a simple direct microscopic examination will produce significant information concerning the number and type of organisms present.	
Standard plate counts are frequently used to give an approximation of the total number of microbes in the sample being tested.	*What provisions does your community or county make to monitor the quality of your food?*
	How is the determination made as to whether shellfish are fit to eat? What effects have our polluted waters had on the shellfish industry?

burrowing animals such as earthworms, gophers, snakes, moles, and mice without realizing that they, and other living things killed by the poisons, enrich the soil in many ways. Along different lines, people destroy many microorganisms by planting single crops which can not be used by many organisms for nourishment. Or, they pave over large areas of soil either because they want to use the land for roads, parking lots and other amenities; or because they don't want to bother with weed-covered vacant lots.

[41]Carbon is released from dead plants and animals through the action of many different bacteria and saprophytic fungi. Each kind of decomposer is equipped to cause the decay of a few of the complex organic compounds found in living organisms, and to release the carbon locked up in these compounds back into the soil.

Phosphorous, which is an important element in the nucleic acids so fundamental to life, is obtained from organic compounds broken down by bacteria and fungi into inorganic orthophosphates, which are absorbed through the roots of a living plant.

[42]Primary treatment plants separate the particulate matter of raw sewage from the water by settling the heavier solids and skimming off the lighter ones. Secondary treatment plants use biological degradation (bacterial action) to treat output from the primary facility. Tertiary plants apply various types of additional treatment to the effluent from the secondary system.

In 1974, of a total of 21,065 sewage plants in the United States, 2,723 were primary treatment plants; 2,575 were adequate secondary plants; 3,287 were inadequate secondary plants, and 10,153 had

data insufficient for classification. Only 975 were tertiary treatment plants. The figures for New York State were respectively: total 747: 472; 34; 66; 135; 16. (See *Environmental Quality* - The Sixth Annual Report of the Council on Environmental Quality, 1975, pp. 444-445.)

For up-to-date information on the status of the Federal Water Pollution Control Act and the Amendments of 1972, students may write to the Environmental Protection Agency, Washington, D.C.

[43]Undesirable microorganisms are destroyed as the cheese ages and the pH tends to become more acid. These cheeses must age a minimum length of time so that producers can be certain that only the desired microorganism is developing. If undesirables take over, there will be definite signs of spoilage within the prescribed length of time.

[44]There are two methods of pasteurizing in current use: (1) Heat the product to about 63°C, maintain it there for 30 minutes—then cool it down. (2) Pump a thin film of the product over a hot metal plate so that it is heated to about 72°C for 30 seconds.

Pupils should understand that homogenized milk is also pasteurized. Homogenization is a process used to break up the fat globules in the milk and has nothing to do with the destruction of pathogens.

[45]Live microorganisms are destroyed when heated to 100°C. However, some spores are able to survive boiling for more than 15 hours. The Clostridium Botulinum organism can withstand boiling up to 5 hours.

Proper use of the pressure cooker at 15 lbs. per sq. in. for 15 minutes will destroy these organisms.

When canned food cools, a vacuum results which also inhibits growth. Any swelling of a can, or a hole in the can which allows air in, or any abnormality observed in the can's contents indicates spoilage, and the food should be discarded.

Botulism can result from eating improperly canned foods. The toxin is absorbed directly from the stomach and intestines and affects the nerves of the body. Paralysis of the face, eyes, throat, and respiratory system ensues. About 25 percent of the people who contract this poison die.

[46]Ultraviolet lamps are in use in many food processing, storage, and handling areas. They are also used in laboratories, hospitals, and schools. These lamps are also used to control surface growth on foods, such as meat, cucumbers, and cheese, while they are being prepared.

Other forms of radiation, such as X-rays, gamma rays, and streams of atomic particles, can also be lethal to microbes. Much experimentation is in progress in the use of these radiations.

[47]Of the many types of bacteria, some grow best at very low temperatures, others at high temperatures. Cryophilic grow best from 15° to 20°C. Mesophilic grow best from 25° to 40°C. Themophilic grow best from 45° to 55°C. There is also a group called thermoduric bacteria which cause a great deal of trouble in milk processing plants and food canning plants. These organisms are capable of withstanding very high temperatures.

APPENDIX - LEARNING ACTIVITIES

C-1. Cell Models [1, 11, 18]

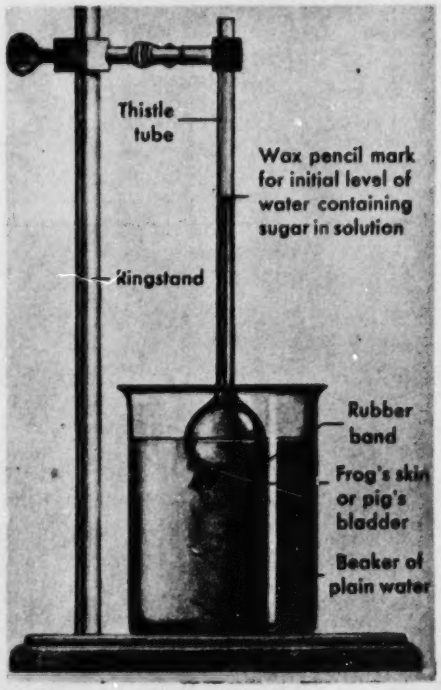
To aid pupils in the visualization of a cell, cell models may be constructed of such materials as clay, papier-mache, or plastic.

To produce a dynamic model, place some nutrient agar inside a form of cellophane wrapping. The cellophane serves as the cell membrane and the agar as a jelly-like cytoplasmic material. Marbles or small chunks of anything available may be inserted to represent the organelles. Different colors may be used for the nucleus and mitochondria. Green peas might become the chlorplasts if a plant cell is being constructed.

When preparing the agar for this cell, a small amount of indicator, such as phenolphthalein, may be added to enable the teacher to show an interesting demonstration of the process of diffusion into the "cell." When ammonium hydroxide is placed near the cell, it will diffuse through the cell membrane and cause the indicator to turn pink.

C-2. Osmosis [3, 1, 5]

To demonstrate osmosis and osmotic pressure, set up a demonstration in which a thistle tube containing a salt or sugar solution has a permeable membrane stretched across its mouth, and is then inverted into a beaker of distilled water. Water from the beaker will flow through the membrane, but the solute will not. The solution in the tube will rise until the osmotic pressure is counterbalanced by the height of the column. A force proportional to the height would have to be applied to a piston in the tube to oppose the rise of the solution. This force is a measurement of the osmotic pressure.


Thistle tube
Wax pencil mark for initial level of water containing sugar in solution
Ringstand
Rubber band
Frog's skin or pig's bladder
Beaker of plain water

C-3. RNA Codons [21, 13]

The combinations which can be made from the four nitrogenous bases of RNA (uracil, guanine, adenine, and cytosine) code the 20 amino acid "building blocks" of protein. When the genetic code was broken by Marshall Nirenberg et al in the 1960's, it became possible to identify the composition of the codon (combination of the four bases) which dictated any one of the amino acids.

Have students fill in the letters of the missing codons in the following diagram on the following page. (For simplicity, the amino acids are designated only by their first three letters.)

second letter

first letter	u	c	a	g	select one third letter
u	uuu phe uuc uua leu uug	—— —— —— —— ser	—— —— tyr —— —— stop —— stop	—— —— cys —— —— stop ugg trp	u c a g
c	—— —— —— —— leu	ccu ccc cca pro ccg	—— —— his —— —— gin	—— —— —— arg ——	u c a g
a	—— —— ile —— —— met	—— —— thr aca acg	—— —— asn —— —— lys	agu agc —— agg	u c a g
g	guu guc gua ual gug	—— —— ala —— ——	—— —— asp —— —— glu	—— —— gly —— ——	u c a g

C-4. Genotype and Phenotype [1, 2, 6, 16]

Every living thing is a product of its heredity and its environment.

To test the effect of environment on a specific trait, set up an experiment involving some fast-growing seeds (radishes, wheat, etc.). Soak the seeds in water overnight and plant them in two pots. Keep both pots in a totally dark place until the leaves have started to develop. Move one pot to the light and leave the other in the dark. After a few days, compare the difference in the color of the leaves. The plants in the light should be green. Those in the dark will probably be pale yellow or white.

Ask students to explain what had probably happened to cause the difference between the plants in the two pots. How could they prove that the plants which were kept in darkness still have the potential for producing chlorophyll?

C-5. "Water drop" Microscope [4, 6, 1, 2]

A "water drop" microscope that will magnify approximately 100 diameters can be constructed as follows:

(a) Remove the crimped ends of an empty metal can with a rotary can opener.

(b) Cut through the can lengthwise with a scissors or tin snips.

(c) Cut the rectangular sheet of metal into strips one inch wide.

(d) Cut off the sharp corners of each strip.

(e) Drive a small finishing nail through the center of each metal strip.

(f) Turn each strip over and file off the ragged points of metal. Insert the nail into the hole again to make it perfectly round.

(g) Bend down slightly the ends of the metal strips.

(h) Find a pocket mirror and lean it against an art gum eraser or small piece of wood.

(i) Support a glass windowpane with books. Place the slanting mirror under the glass pane and one of the prepared metal strips directly above it.

A microscope made in this way is extremely easy to use and gives approximately the same magnification as the low power of a compound microscope. A sufficient number of the metal strips should be made so that each pupil has one or more. Pupils should be encouraged to set up microscopes and use them at home.

The following simple directions should be observed in using the "water drop" microscope:

(a) Place the microscope on a desk or table and adjust the mirror so that light from the sky is reflected directly upward through the pane of glass.

(b) Dip a sharpened pencil into a glass of water and shake off any large drops. Place the point of the pencil into the hole of the metal strip so that a fairly large drop of water is deposited directly over the hole. Several trials may be required before the drop is round and perfectly centered.

(c) Place a drop of the material to be observed on the glass pane and spread it slightly.

(d) Slide the metal strip containing the water drop into position directly over the material to be observed.

(e) Place one eye very near the water drop and press down on the strip with one finger until the object comes into focus.

C-6. Basic Microscopic Technique [4, 2, 11]

Have students sketch the parts of a compound microscope and explain their function. After diagraming the parts of a microscope and learning the general rules for its use, have students:

(1) Use a piece of lens paper to clean the objective lenses, and then the eyepiece or ocular. If the lenses are still very dirty, suggest breathing on them and wiping with lens paper. If the dirt is still

there, try a piece of lens paper moistened with Xylol or 95 percent alcohol. Have the students clean the mirror last.

(2) Be sure that the low power objective is clicked into place.

(3) Adjust the mirror while looking through the eyepiece so that enough light is passing through. Blue tint microscope lights are recommended, but light reflected from the blue sky is satisfactory. Caution students NOT to focus on the image of the sun. Severe burns of the retina may result in permanent blindness. Also, manipulate the light adjustment to let the maximum amount of light enter the objective.

(4) Place a slide on the stage. For beginning observations, newsprint letters are frequently used. However, prepared slides or some of the pupils' own cheek cells may prove more interesting to them.

(5) Lower the objective as far as possible and then, while looking through the eyepiece, raise the objective slowly until the object is in focus (use the coarse adjustment knob to begin with and the fine adjustment knob for more precise focusing).

(6) Have pupils sketch what they see. Have them date and label all sketches.

(7) Have students move the slide forward and note whether the image moves forward or backward. Have students move the slide to the right - to the left. What is the observed effect of these movements? If the observer sees an object moving in a clockwise direction, is the object on the slide moving in a clockwise or counterclockwise direction?

Pupils should observe a number of different slides while using low power, before being instructed in the use of high power. To use high power, have the pupils:

(1) First, while using low power, center the object being examined. It is important to locate and center the object under low power because the total field in view under high power is much smaller than that under low.

(2) Carefully rotate the high power objective into place while watching to see that it does not hit the slide. If it is going to hit, raise the tube slightly.

(3) Adjust the mirror to increase the light being transmitted through the object.

(4) The high power objective focuses very close to the object. The objective must be raised very slowly (using the fine adjustment knob only) while focusing.

(5) Draw what they see.

If pupils are unable to locate the object under high power, have them switch back to low power, locate and center the object, and try again.

Efficient use of the microscope requires that both eyes be kept open in order to reduce strain from long periods of microscope work.

C-7. Simple Slide-Making Techniques and Staining [4, 6]

(a) Simple wet-mount slides are temporary because the mounting fluid evaporates. The mounting fluid may be a drop of water, salt solution, or stain.

Have students place a drop of fluid in the center of a slide, put the specimen in the fluid, and add a cover slip without trapping air bubbles. Care must be taken in applying a cover slip. Place one end in the drop, and slide the cover slip down gently. If the cover slip is applied too quickly, there will be air bubbles under it.

When observing onion cells, students should take a very thin slice from one of the inner surfaces of the onion and mount it in a drop of Lugol's solution in order to stain the parts of the cells.

Epithelial cells are also easy to prepare by this method. Have students take a clean toothpick and scrape the flat end over the inside of the cheek; place the scrapings in a drop of Lugol's solution; and, apply a cover slip.

Additional stain or fluid may be added by placing a drop alongside the cover slip. Capillary action will cause the fluid to flow beneath the cover slip.

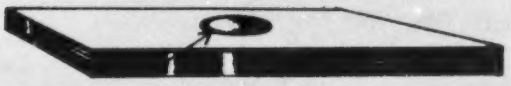
(b) Hanging drop technique for direct observation of living bacteria, yeasts, some algae, and protozoa. (See diagram below.) Have students:

(1) Place a small amount of vaseline around the rim of the depression of a concave or deep well slide.

(2) Carefully put a loopful of the liquid to be observed in the center of a cover slip.

(3) Invert the slide so that the depression is placed over the drop. Press the slide down in order to make the vaseline adhere to the cover slip.

(4) Gently, but rapidly, turn the slide upward and place it on the stage. The drop should be hanging from the cover slip, and observations of living and moving forms may be made.



Concave Slide

Cover slip with specimen centered

Slide inverted over cover slip

Hanging specimen for observation

(c) Smear technique. Students may use this method to prepare slides of many different materials.

If making a slide of liquid material, put a drop on a clean slide.

If the material is solid, but soluble, put a drop of water on the slide and use a wire loop to mix the material thoroughly. Spread the liquid out in a thin film on the slide. A common error is to have the smear too thick to allow the light to pass through it. Let the smear dry in the air.

Fix the smear by quickly passing the slide, smear side up, through a bunsen flame two or three times.

The smear is now ready for observation or for staining if desired.

(d) Simple staining. There are many organic compounds commonly used as stains. The three most commonly used when working with microorganisms are methylene blue, carbol fuchsin, and gentian violet (also known as crystal violet). Formulas for stains may be found in *A Sourcebook for the Biological Sciences.*

After a smear is fixed on the slide, a stain will usually be applied in order to outline the cells more clearly. Staining is an art and practical experience is the only way to become proficient at it. For the purposes used here, a drop or two of stain will be put on the smear and allowed to stay for 1 to 3 minutes. Then the excess stain is washed off under gently flowing water.

It is difficult for the beginner to make a clearly stained smear. If the dye is left on too long, the field will be too dark for observation. If the smear is not properly fixed or if it is washed too vigorously when removing the excess stain, the entire smear may be washed away.

C-8. Making Microscopic Measurements [5, 4, 6]

The use of the microscope to make size measurements in metric units should be encouraged and practical to the fullest extent possible. Not only does this help pupils to better realize the size relationships of the objects being observed, but such procedures encourage the development of careful observational skills.

Although ocular and/or stage micrometers may be used, most school laboratories will not have them. Pupils can gain an understanding of measuring techniques if simple, everyday equipment is employed. The simplest procedure involves the approximation of the dimensions of objects by means of diameters of the microscopic fields used.

To measure the diameter of the low-power objective field, place a transparent plastic ruler on the stage with its edge across the center of the field. By focusing carefully, the field diameter can be measured directly in millimeters. Conversion to microns can then be accomplished by using the metric equivalents 1 mm = 1000 μ.

The diameter of the high-power objective field may be estimated either by direct observation (using the same method as with the low-power objective field), or by means of ratios. For example, typical total magnifications using 10X ocular lenses are often 100X (10X x 10X) for low power, and 430X (10X x 43X) for high power. The ratio would thus be 1 divided by 4.4 (22.7%) that of the low-power field.

To estimate the size of a real object, note carefully its relationship to the diameter of the field of view being employed. If, for example, the diameter of a cell being observed is one-third that of the low-power field, and you have calculated the diameter of the field as being 1600 μ, then the diameter of this cell would be approximately 540 μ.

C-9. Observing Pond Water [1, 2]

Have pupils bring samples of water from ponds, rain puddles, or stagnant pools around their homes or school. To insure that microorganisms will be available for observation, begin a hay infusion about two weeks before needed. Add some dried grass, lettuce, or straw to a large jar of pond water. Cover the jar loosely. If the water level decreases, add distilled rain water or pond water to the jar. (Tap water might kill the microorganisms present.)

C-10. Where Microbes Are Found [1, 2, 4, 6]

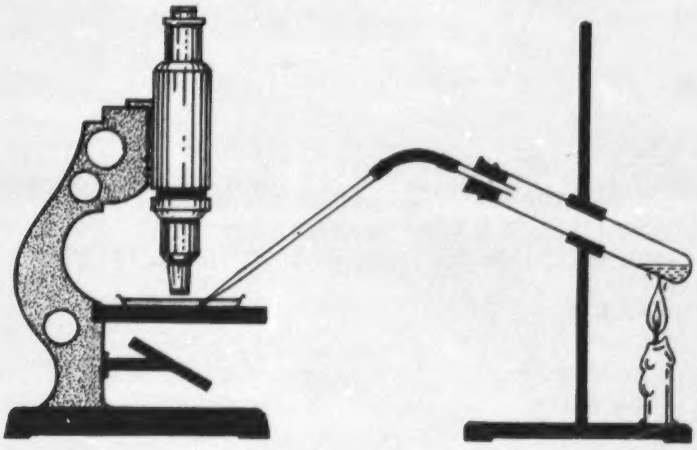
Bacteria and other microbes are found nearly everywhere. When provided with the materials and conditions needed for growth, each microbe may multiply until it forms a mass called a colony, which is usually large enough to be seen with the unaided eye. Although it is usually more convenient to culture organisms on nutrient agar in petri dishes, it is recommended that the first cultures to be used in this unit be grown on orginary food materials. This will make it clear to pupils that common food materials provide suitable nutrients for microbe growth.

Set up a series of large culture dishes (plastic food containers or cottage cheese containers will work well) with such materials as: (1) hay or dried grass, with water added; (2) dried beans or peas, with water added; (3) moistened bread which has been wiped over a dusty surface; (4) peppercorns, with water added; and (5) decaying fruits or vegetables of any kind.

Cover all dishes, and place them in a warm place, away from direct sunlight. Have pupils make careful observations of the dishes daily for about 5 days, keeping complete records of any changes which may be observed.

Pupils should be encouraged to observe bits of the visible growth with a microscope and to record their observations. The teacher may wish to prepare temporary mounts for observation. There will be much opportunity for individual observational work as this unit progresses.

C-11. Effect of Chlorine on Microbes [1, 4, 5, 8]



Chlorination is the standard method for destroying pathogenic microbes which might be found in public water supplies being provided for human use.

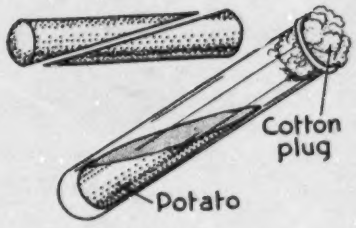
Pupils can demonstrate the effectiveness of this process. Place a shallow dish of a mixed media culture of protozoa on the stage of a microscope or microprojector; observe their motion. Set aside half of the culture as a control. Assemble a simple chlorine generator consisting of a Pyrex tube and a delivery tube, drawn out to a fine tip, as shown in the illustration. In the test tube place a pinch of manganese dioxide and 0.25 ml of concentrated hydrochloric acid. Place the delivery tube into the culture, and gently heat the test tube. After 2 minutes, remove the delivery tube before turning off the heat, and place it in a solution of hypo (sodium thiosulfate) to neutralize any excess chlorine. Observe the organisms again to check their activity. Place the control sample back on the microscope to make a comparison. The demonstration can be set up with the microprojector so that the entire class can watch the experiment.

C-12. Preparation of Culture Media [6, 4, 14]

(a) Homemade media. Before the development of the petri dish and standard nutrient agars as culture media, pioneer bacteriologists grew bacteria on such materials as potatoes and in various types of broths. These provided a source of food and a place where the bacteria could grow. Pupils might like to repeat some of the early methods.

(1) Potato medium. Cut a cylindrical piece of potato with a cork borer or apple corer. Halve the cylinder with a diagonal cut as shown in the diagram. Leave the pieces of potato in cold running water for from 3 hours to overnight to reduce their acidity.

Place pieces in tubes with the pointed end uppermost as shown in the diagram, add 3 ml of distilled water to each tube, and stopper the tubes with non-absorbent cotton. Sterilize the tubes in a pressure cooker at 15 pounds pressure for 30 minutes. It is also possible to use slices in petri dishes where this seems more convenient. Slices of other materials such as carrots may also be used.



Pupils may expose these tubes or plates to the air, or inoculate them by touching them with a platinum or nichrome wire laden with bacteria or a dust sample. Incubate the plates or tubes in a warm, dark place for several days, during which time colonies may develop.

(2) Infusions. A wide variety of infusions can be prepared be heating materials for a few minutes in an appropriate amount of distilled water, and then straining them through cloth and/or paper. Some materials which can be tried include white potato, white beans, dried hay, hamburger (lean meat is better). If solid media are desired, nonnutrient agar is added at the rate of about 20 to 30 grams per liter of hot broth. On cooling, the medium will solidify, so it must be poured into plates or tubes before it cools below about 42°C. If the infusion is to be used as a liquid medium, it is simply placed in tubes, plugged with nonabsorbent cotton, and sterilized.

(3) Preparation of a sterile nutrient agar. Weigh out the following materials and then combine them with one liter of distilled water:

Peptone	10 gm
Salt (NaCl)	5 gm
Beef extract	3 gm
Agar	30 gm

Heat the peptone, salt, beef extract, and water slowly to 45°C, being certain to stir until all materials are dissolved. Add the agar and heat slowly until this is also dissolved. Adjust the pH to about 7.5 by adding small amounts of sodium bicarbonate and checking with litmus paper. Autoclave at 15 pounds pressure for 15 minutes, and pour into sterile bottles, tubes, or plates.

(b) Standard media. Standard media in many forms may be purchased from supply houses. These range from prepared sterilized test tubes of agar media or broths to dehydrated media which are to be prepared in the laboratory. In order to involve students to the fullest extent possible, it is usually more desirable to obtain dehydrated media and then have pupils make the preparation. Full directions for the preparation of these materials are found on the package. A recent innovation is preweighed quantities of the dehydrated media. These can save much time, but their use prevents the involvement of pupils in weighing out the necessary amount of the dried material. Their use

should therefore be limited to the preparation of media by the teacher, where the saving of time is important.

C-13. Optimum Conditions for Bacterial Growth [3, 1, 4]

Optimum growth of bacteria is achieved under rather rigid conditions of temperature, moisture, light, and availability of food and oxygen. The necessity for the first four of these conditions can be demonstrated by the following experiments.

An easy way to inoculate a number of culture plates with approximately equal numbers of bacteria is simply not to sterilize either the nutrient, agar, or the petri dishes. Spores found on the glassware and falling from the air will do a good job of inoculation. Prepare eight plates of nutrient agar in this manner. As soon as the agar has solidified, the plates must be kept refrigerated until used. They should not be prepared more than 2 days prior to use, for there may be some slight growth of bacteria under refrigeration.

Heat 1.5 gm of powdered plain agar in 100 ml of water until dissolved. Pour some of this into an unsterilized petri dish and refrigerate until used. This will be inoculated the same as the others, but contains no food.

(a) Temperature. Place one inoculated petri dish of nutrient agar in an incubator, another in the food compartment of a refrigerator, and a third in a dark drawer at room temperature. The only variable is that of temperature, all other conditions remaining constant. Examine daily for 2 or 3 days and compare the visible growth in each.

(b) Moisture. Remove the cover from one inoculated petri dish of nutrient agar. This will allow it to dry out rapidly so that moisture is not available to the bacteria. Place the opened dish, together with a covered one for control, in an incubator. Examine both dishes daily for 2 or 3 days and compare the visible growth in each.

(c) Light. Place one inoculated petri dish of nutrient agar in a dark drawer at room temperature and another under an electric lamp. Examine daily for 2 or 3 days and compare the visible growth in each.

(d) Availability of food. Place the inoculated petri dish of plain agar and one of nutrient agar in an incubator. Examine daily for 2 or 3 days and compare the visible growth in each.

C-14. To Multiply Microbes Found in the Air and on Everyday Objects [3, 6, 12]

Prepare about 10 petri dishes of sterile nutrient agar.

- Leave each in a warm place for 24 hours to be sure it is sterile; non-sterility is indicated by the appearance of bacterial or mold colonies.

- Expose four or five of the plates to the air in various locations by removing the cover for 5 to 10 minutes. Such places as the locker rooms, the cafeteria serving line, the classroom, or the corridor during a passing period may yield interesting results. Label the plates, indicating where, when, and for how long exposed.

- Touch common objects lightly to the surface of the agar. Such objects as pencils, combs, coins, doorknobs, fingers, hair, or books may be used. Label these plates to indicate what objects were used to touch the agar.

- Save one plate as a control.

- Leave the plates for a day or two in a warm, dark place such as an incubator or a warm drawer or closet. Growth is slower at room temperature than at body temperature or in an incubator.

- After 2 days, observe the plates and count the number of colonies on each. Remember that each colony usually indicates a single bacterium originally placed on the agar. Keep the plates closed during the observation. Record the number of colonies on each, as well as their general characteristics. Repeat this procedure for the next few days, noting especially any further changes which can be observed. If additional colonies can be found, it is probable that at the time of the previous count, they were too small to be seen. Some bacteria multiply much more slowly than others.

- Do not open these plates while counting the colonies. Autoclave plates (cultures and all) before disposing of them.

C-15. Making Bacterial Smears [6, 1, 4, 14]

The teacher should allow the pupils to practice this technique only with nonpathogenic microorganisms. Care must be taken because outside contamination is very difficult to prevent.

Students should be especially careful to flame the inoculating loop before and after every use. Never place a contaminated loop on the table. Never use a loop contaminated by the table top to remove the bacteria. When removing bacteria, leave the culture uncovered as little as possible. Lift the cover of the petri dish on one edge only and insert the loop. Do not leave it open longer than is absolutely necessary. Have pupils hold their breath when the dish is open. Thus the pupils will avoid inhaling contaminants from the culture and exhaling microbes which may inoculate the culture with a different organism.

The following procedure is recommended.

With an inoculating loop, put a drop of water in the center of a clean microscope slide. Flame the loop until red hot, and then allow it to cool for use. Use the loop to remove a minute amount of material from a colony of bacteria by touching it lightly to the colony. Stir the bacteria into the drop of water, making a thin, even film on the slide, and then allow it to air dry. Sterilize the loop before putting it down once again. When the slide is dry, pass it, bacteria side up, through the flame two to three times to "fix" the bacteria to the slide.

Place the slide with a fixed bacteria across the top of a beaker and cover the smear with several drops of crystal violet stain for about 1 minute (if methylene blue stain is used, increase the staining time to about 3 minutes).

Tilt the slide so as to pour the stain into the beaker, and then rinse the slide under gently running water until all excess stain has been removed from the slide. Examine the slide under low and high power of the microscope. If the bacteria have been spread thinly over the surface of the slide, it should be easy to determine the shape of the individual cells.

If a permanent slide is desired, place a drop of Canada balsam on the film of bacteria and then press a cover glass on the surface of the slide, using enough pressure to cause the balsam to completely fill the space between the slide and cover glass. Allow to dry in a horizontal position for 10 days to 2 weeks before using the slide.

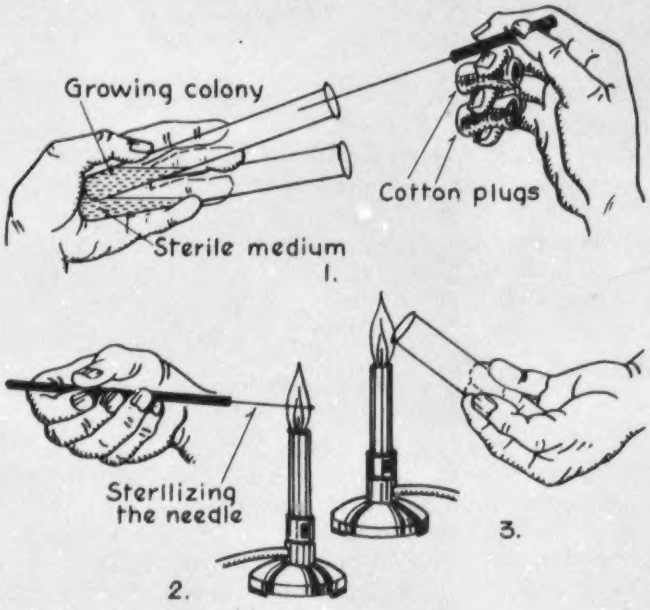
Oral bacteria. A drop or two of saliva or scrapings from around the base of the teeth can be used as sources of the three major groups of bacteria. If saliva is to be used, allow a drop of two to dry on the center of a clean microscope slide after spreading it around with either a toothpick or an inoculating needle. If scrapings are to be used, the material is removed from around the base of the teeth with a toothpick and is then spread on the center of a clean slide in a drop of water. When dry, smears from either of these sources are then fixed and stained as indicated above.

Bacterial smears may also be made from such foods as fresh sauerkraut juice, cultured milks, and cheeses.

C-16. Techniques of Bacteriology (Aseptic Techniques) [6, 1, 3, 4]

If pupils are going to do more advanced lab work at this point, the techniques of handling and growing microbes must be mastered. Although they may seem complex at first, the techniques are really quite simple to learn, and with a little practice, most pupils will develop competence in a short time.

IMPORTANT: All work of this type should be done with nonpathogenic microorganisms, such as Bacillus subtilis, Spirillum rubrum, or Sarcina lutea.



(a) How to transfer bacteria. Basic to all operations in aseptic technique is the transfer technique. The diagram illustrates how to hold the transfer needle and tubes. Hold the two tubes, one with the colony actively growing [preferably no more than 24 hours old] and the other, a sterile one, in the left hand. Place the needle in the right hand. Sterilize the needle by holding the wire portion at the tip of the blue portion of the flame and heat

it until it glows orange over its entire length. Be sure to heat the part of the handle that may touch the tip of the tubes. Allow the needle to cool momentarily before transfer while the plugs are being removed from the tubes.

Remove the cotton plugs with the fingers of the hand holding the inoculating needle. Flame the mouths of the tubes by passing them through the flame twice. Remove a bit of the bacterial culture from the surface of the agar and immediately transfer it to the surface of the sterile medium. Spread this material out to insure good growth, but do not dig into the agar. The pressure of the weight of the needle is sufficient to apply the inoculum. Flame each tube's mouth again and put the cotton plugs back in position. Flame the inoculating needle carefully to destroy all remaining material.

(b) How to isolate a pure culture. While the bacteria we find in nature are usually found growing together, mixtures of bacteria are usually not desirable in laboratory work. The separation of one type of bacteria from other types is known as growing the bacteria in "pure culture." The techniques for isolating pure cultures were first devised by the German bacteriologist, Robert Koch, about one hundred years ago, and the techniques used are virtually unchanged today. A method commonly employed to isolate pure cultures is the streak-plate method.

Streak-plate method. This method makes use of prepoured, sterile petri dishes containing a medium suitable for the growth of the organisms to be cultured.

Flame an inoculating loop to sterilize it, and allow it to cool briefly. Use it to remove a drop of bacterial suspension from a broth, an infusion, or other bacterial culture. Raise the lid of a petri dish high enough to insert the wire loop with the drop of the culture, but no higher than necessary. Carefully streak the inoculum according to the diagram shown above. Touch the surface lightly so that you do not break into the surface of the agar as you streak.

Incubate the plate for several days at 37°C. or for a longer period at room temperature. Locate colonies that are well-isolated, using a hand lens if necessary. Use a sterilized inoculating needle to transfer some material from such colonies to another prepoured sterile petri dish in order to further isolate each culture.

(c) How to differentiate bacteria by using the Gram staining technique. Whereas simple staining methods cause bacterial cells to look different than their environment, differential staining methods seek to distinguish between different types of bacteria. This is possible because microbes differ from one another chemically and physically, and as a result, they will react differently to a given staining procedure. The Gram stain procedure

helps us to divide bacteria into two groups. The first of these includes those bacteria which are stained *purple*. These bacteria are termed gram-positive. The second group, called gram-negative, are bacteria which are stained *pink* by this procedure.

Prepare smears of bacteria according to the usual procedure. After the slide has been allowed to dry and has been fixed, place it on a staining rack or across the top of a beaker. Cover the smear completely (flood it) with crystal violet staining solution and leave the stain in contact with the smear for about 1 minute. Pour off the excess stain, and rinse the slide with water.

Cover the smear with Lugol's iodine solution, allowing it to act for about 1 minute. Rinse the slide with water. Use 95 percent alcohol to decolorize the stained smear by rinsing the stained area with the alcohol until no more blue color flows away - this takes about 30 to 60 seconds. Rinse the smear with water. Blot dry.

Add the safranin counterstain, keeping it in contact with the smear for about 30 seconds. Rinse with water, and blot the slide dry. Be sure to blot, and not rub, the slide to dryness. The slide is now ready to examine with the microscope.

C-17. How Pathogenic Bacteria Are Spread [3, 4, 6]

Pathogenic bacteria are spread in a number of ways, some of which can be demonstrated in the laboratory. In these exercises, only the fact that bacteria are spread by various methods is indicated. Since there is no simple way to determine whether the bacteria found on the cultures are pathogenic or not, extreme caution is essential.

(a) Bacteria spread by droplet infection. Prepare several sterile petri dishes of nutrient agar. Have pupils cough into the plates — a separate plate for each pupil. Incubate and observe for bacterial colonies. Do not open plates while making observations.

(b) Bacteria spread by flies. Prepare a sterile petri dish of nutrient agar. Capture a fly and put it in the plate for several minutes so that it may walk around on the surface of the agar. Remove the fly and incubate the plate. Then observe for bacterial colonies.

(c) Bacteria spread by contaminated water. Sterilize a small screwcap jar by placing it in an oven for 1 hour at 177°C. Use it to collect some water from a stagnant pond or polluted stream. Pour a little of the water onto the surface of sterile nutrient agar in a petri dish. Swirl the plate gently so that the water covers the surface and pour off any excess. Incubate the dish for a day or two and examine for bacterial colonies.

Do not allow pupils to open these dishes. When they are finished, autoclave them before disposal.

C-18. Culturing and Observing Bacteria of the Skin [1, 2, 4, 6]

To gather bacteria found on the skin, it is convenient to use a sterile swab. These may be prepared in the school laboratory, or they may be

purchased in a ready-to-use form. Some prepared swabs, such as those made with calcium alginate wool, have certain advantages over the homemade ones, but those which students might prepare will work satisfactorily. The chief advantage of the alginate swab is its ability to dissolve completely in salts of certain organic acids. Such swabs, sold under the trademark "Calgiswab," are available through most scientific supply houses.

Homemade sterile swabs may be prepared as follows: wrap a small amount of absorbent cotton about the tip of a small stick, such as an applicator stick available in drug stores. The cotton will stick well to the wood if it is moistened with a little water on the fingertips as you twist the cotton onto the tip of the stick. Place two of these swabs in a test tube with a little distilled water, plug the tubes, and autoclave them. This procedure provides sterile, moist swabs which are useful to pick up microbes from the skin and other surfaces.

Bacteria of the skin many be cultured on agar in petri dishes or in broth or simultaneously in both media. Prepare sterile media in accord with the design of the demonstration and in sufficient quantity to allow for a satisfactory number of control plates and/or tubes.

Using the usual aseptic technique, remove the swab from the tube (or package) and then swab a small area of the skin. Immediately thereafter, streak the surface of a plate of sterile nutrient agar. If a broth culture is preferred, use aseptic technique to "rinse" the swab in the sterile broth. If the alginate swab is used, it may be rinsed instead in a broth of medium containing 1 percent sodium citrate or Calgon, in which case the entire swab will dissolve completely. The alginate technique is more convenient if it is desired to make counts of the total number of bacteria collected by wiping a measured area of skin surface, since *all* bacteria so collected will be transferred to the medium. Remember to avoid placing used swabs on counter tops, etc. — you must always make the assumption in bacteriology that exposed materials may contain dangerous pathogens. A convenient method of disposing of the swab is to place it directly into a container of tincture of green soap or some other disinfecting agent.

Incubate the plates and/or tubes so inoculated for a sufficient time for growth to proceed, at which time the desired observations should be completed. For plate cultures, these observations can include colony counts, observations of colony characteristics, and comparisons of the types of colonies noted. *Do not* open the plates and be certain to autoclave them before disposal.

C-19. Phagocytes Engulf Bacteria (Teacher Demonstration - NOT Pupil Activity)

Make permanent slides of some pus from a pimple or boil gathered with a sterile inoculating needle. Exercise great care when handling material of this nature. Maintain sterility. Stain with crystal violet or Wright's blood stain, put a drop of Canada balsam over the completed slide, and apply a glass cover slip. Gently press the cover slip down to force the balsam to spread out under the entire cover slip. Store in horizontal position for 10 days to 2 weeks until the balsam has hardened. Examination under the microscope will reveal bacteria being engulfed by phagocytes.

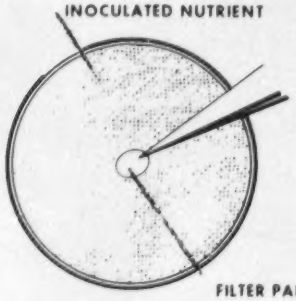
C-20. Inhibition Assay Testing of Antibiotics [11, 6, 13, 1]

The inhibition assay represents a general method for testing the ability of a number of agents to retard bacterial growth. The procedure may be used for testing the effectiveness of varying concentrations of a single antibiotic, comparing the relative effectiveness of several different antibiotics or comparing the sensitivities of different species of bacteria to a particular antibiotic. Only slight modifications of procedure are necessary to adapt the general procedure to that needed for a particular test.

The general procedure depends upon providing an agar medium which has a uniform distribution of bacteria. This may be accomplished in several ways. Perhaps the easiest method involves preparing tubes of sterile agar which are inoculated with bacteria while still liquid. The bacteria may be a pure culture of one of the harmless kinds, such as *Bacillus subtilis, Serratia marcesens,* or *Bacillus lactis.* It is important to swirl or agitate the melted agar mixture thoroughly in order to provide uniform distribution of the bacteria. Pour petri dishes with this medium. If the dishes are not to be used immediately, they must be refrigerated as soon as the agar has hardened. In any event, they should not be prepared more than 2 days prior to use.

In an alternate method of providing a uniform distribution of bacteria on each plate, the surface of the agar is streaked over its entire area with a broth culture of bacteria. Sterile swabs are very useful for this purpose.

Paper discs which are to be saturated with the drug to be tested can be easily prepared. Cut out round discs of blotting paper with a paper punch. Place a number of such discs in a tube and sterilize them by autoclaving. With sterile tweezers, dip a disc into some of the drug to be tested. After the surplus has drained off, gently but firmly place the disc on the surface of the agar. Do not press strongly enough to break the surface of the agar. Three or four discs can be placed on a single petri dish, provided that they are evenly spaced. It is also possible to purchase commercially prepared antibiotic discs. Where more critical work is desired, these prepared discs are imperative, since they are impregnated with a known amount of antibiotic.



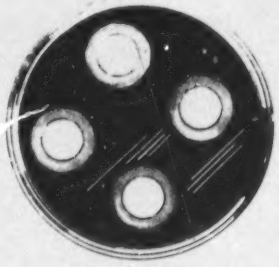
If homemade discs are to be prepared, it is suggested that the following fairly typical antibiotic solution be used.

Penicillin - - 100,000 units in 10 ml of sterile distilled water.
Streptomycin - 0.5 gm in 3 ml of sterile distilled water.
Terramycin - - 0.5 gm in 3 ml of sterile distilled water.

Be certain to prepare a control disc for each dish. This may be done by soaking the control disc in sterile distilled water and placing it in the petri plate in the same manner as the others.

Incubate the plates for 2 or 3 days, examining them daily. Where the bacteria grow, a general cloudiness will be evident, for the bacteria have been seeded throughout the agar, not just on the surface.

Around the paper discs, the material being tested diffuses into the agar, being more concentreated in the agar near the disc than farther away. Where inhibition has occurred, there will be a "zone of inhibition" — a clear zone — around the disc. The width of this clear zone is a measure of the effectiveness of the antibiotic material being tested.



C-21. Infectious Diseases in Plants [2, 13, 4, 1]

We usually associate infectious diseases with animals and often tend to forget that plant diseases are of great economic importance. It is possible to determine infection in plants with viruses.

(a) Tobacco mosaic virus. Most of the commercial forms of tobacco are infected with the tobacco mosaic virus. This virus is very stable and remains active in cigarette, cigar, and pipe tobacco.

Plant tobacco seeds in clay pots or flats according to directions obtained from the seed distributor. If tobacco seeds or plants cannot be obtained, tomato seedlings may be used instead. Tobacco plants are ready to inoculate when they have six to eight fully expanded leaves. One-half of each leaf can be used for the experiment, leaving the other half for a control area.

Grind a small amount of tobacco in a few drops of water, using a mortar and pestle. Sprinkle a little 400-500 mesh carborundum powder on a leaf. Wet a forefinger in the tobacco water and, supporting the underside of the leaf with the other hand, rub the wet forefinger on the carborundum-sprinkled upper surface of the leaf. This will produce microscopic breaks in the leaf cells and force virus particles into the cells. Flush off the surface of the leaf with water after rubbing. Observe the plants over several weeks. If tobacco mosaic disease is present, small round black spots will appear on the leaf surface.

(b) A latent virus in potatoes. Many varieties of potatoes carry a virus in a latent condition. In this condition it has no visible effect on the potato, but can be inoculated on potato leaves where it will produce characteristic lesions.

Half-submerge several potatoes in wet sand to sprout. When they begin to produce leaves, place several other potatoes in damp sand to sprout. When the second potatoes begin to sprout, grind several of the shoots or sprouts in a little clear water with a mortar and pestle. Inoculate the leaves near the growing tips of the other potatoes, using the method described in (a) above. Some plants should be left uninoculated to serve as controls.

The leaves of young pepper plants grown from seed can also be inoculated and the latent virus from the potatoes, if present, will grow in their cells.

About 2 weeks after inoculation, the new growth will develop a matted, distorted type of growth if the virus is present.

(c) Other possible experiments.

(1) Effect of temperature on virus. After grinding the infected plant tissue, put the virus extract in a test tube in a water bath at a variety of maintained temperatures, up to the boiling point for identical lengths of time, before using it for inoculation. Freeze some of the extract before using. What effect does temperature have upon these viruses?

(2) Effect of dilution of virus. Make various dilutions of the virus extract obtained by grinding infected plant tissue. Use these dilutions for inocuation.

(3) Effect of using an abrasive such as carborundum in inoculation. Inoculate some plant leaves with virus extract without using the carborundum and some using the abrasive.

(4) Effect of different methods of inoculation. Use such materials as cotton swabs, brushes, or needles, as well as your forefinger, to inoculate leaves. Use each with and without the carborundum. Which method seems to be the most effective? Ask pupils to form an hypothesis as to why one method is more effective than another.

C-22. Demonstrating the Presence of Microorganisms in Soil [2, 1, 8]

Collect soil samples in sterile, covered containers. Prepare sterile petri dishes of nutrient agar. Carefully dot the dishes (very lightly) with soil be means of a sterile inoculating needle. Incubate for 24 to 48 hours, and count the number of colonies on each plate.

Soil bacteria are both numerous and vital to the maintenance of fertile soil for plant life and to carry on the decay process. The samples grown in the lab will probably contain nonpathogenic organisms.

Two dangerous microbes are found in the soil. These are: *Clostridium tetani* - a bacillus which causes lockjaw or tetanus, and *Clostridium perfringens* - an anaerobe which causes gas gangrene. Both organisms are anaerobic and therefore would not grow in the laboratory culture described above. Pupils should learn that wounds which allow these organisms to penetrate deeply are very serious.

C-23. Foods from Microorganisms [14]

Determine which of the foods in the menu below were partially made by the action of microorganisms.

Appetizers

Olives Carrot Sticks Sour Pickles
Tomato Juice
Cantaloupes

Entrees

Roast Pork
Chicken in Wine Sauce
Broiled Trout

Vegetables

Mashed Potato or Baked Potato with Sour Cream
Peas
Carrots
Sauerkraut

Salads	*Dressings*
Heart of Lettuce	French
Peach and Cottage Cheese	Blue Cheese

Hot Garlic Bread with Butter

Desserts

Apple Pie
Chocolate Layer Cake
Crackers and Roquefort or Wine Cheddar Cheese
Raspberry Yogurt

Beverages

Coffee Tea Milk Buttermilk Cider Beer

C-24. Observing Yeast Cells and the Production of CO_2 [1, 2]

(a) Take a small piece of a yeast cake, dissolve it in water, and place a drop of this material on a clean slide. Add a drop of Lugol's solution in order to stain the cells for easier viewing.

If the yeast is allowed to sit overnight in a solution of molasses and water, the pupils may be able to observe the budding of yeast cells. Do not use Lugol's solution since it kills the cells.

(b) Place a solution of yeast, water, and molasses in a flask. Use a one-hole stopper with a glass tube in it to close off the flask. Attach a piece of rubber tubing and insert the other end in a tube of clear limewater. As

fermentation occurs, the limewater will turn milky due to the carbon dioxide which is released.

C-25. To Grow a Variety of Molds [1, 2, 9]

Line the bottom of petri dishes or plastic containers with moist filter paper and place a sample of bread, orange, cheese, or jam in each - one food to a container. Place these dishes in a dark closet at room temperature for 48 to 72 hours. Observe the molds which have developed on each food. Note differences in the type or color of the growth on each of the substrates.

An interesting extension of this lab would be to use an inoculating needle to transfer some mold from an orange to a piece of unmolded bread. Also try transferring a bread mold to an unmolded orange. Put them in a dark closet for 48 to 72 hours and observe.

C-26. Bacterial Actions on Food [1, 2]

Use the following information for classroom discussion purposes.

Food	Comment
Sauerkraut	Cabbage is cut into thin layers. Salt is added to draw out the juices which are rich in sugar. Lactic acid bacteria, which are always present, then ferment the sugar and sauerkraut is formed.
Fermented Milk	Fermented milks are such products as buttermilk, yogurt, and acidophilus milk. They are produced by the action of bacteria on milk from cows, goats, mares, camels, yaks, sheep or llamas. Buttermilk is made by adding a form of streptococcus. Yogurt and acidophilus result from the action of a lactobacillus. Milk contains many of these organisms normally but, if allowed to sour on its own, microbes which will spoil the food may take over. Commercially, these fermented milk products are made from pasteurized milk in which any pathogens present are destroyed. Following this process, a small amount of the desired organisms is introduced to start the fermentation process. (See Activity C-27.)
Butter	A strain of streptococcus is responsible for the flavor and aroma of butter.
Cottage Cheese	Cottage cheese results from the action of lactic acid bacteria which are added to pasteurized milk. When the cheese reaches a certain point in ripening, the growth of the microorganisms is stopped and the product is cooled and packaged.
Cheddar Cheese	Cheddar cheeses are made from the curd of soft cheeses which is dried and allowed to age a long period of time.

C-27. Standard Plate Counts [6, 5, 2, 1]

(a) Calculation of dilutions. Before doing a standard plate count, the pupils should practice the use of pipettes and the calculation of dilutions.

If the standard volumetric measuring pipettes are used, care must be taken to practice with harmless liquids. If the teacher desires, 1 ml dropper pipettes are available.

Have pupils put nine milliliters of water in each of three test tubes (using a graduated cylinder). Have pupils add one milliliter of a colored liquid such as food coloring to one of the tubes. Shake the tube gently. There will then be 1 ml of food coloring to 9 ml of water. The total quantity of solution is 10 ml . Thus the food coloring is one part of the 10 part solution making a 1/10 (.1) dilution.

Next, remove 1 ml of this dilution and add it to one of the other test tubes containing 9 ml of H_2O. This will be a dilution of 1 to 10 of a 1/10 solution evenly. Perform the operation once more producing a dilution of 1/1000 or .001.

(b) Counting bacteria in milk, milk products, and water. To estimate the number of bacteria in a given milk sample, prepare a petri dish of sterile agar. Using a pipette, add 1 cc of milk directly to the agar. Rotate the petri dish gently on the table to spread the milk over the entire surface. This culture may then be placed in an incubator for 24 to 48 hours or left at room temperature for 4 or 5 days.

A simple way to count the number of colonies developed is to divide the dish (by drawing lines on the cover) into eight secions. Count two or three representative sections, average them, and multiply by 8. The resulting number will be an approximation of the number of bacteria found in 1 cc of that particular sample.

Professional bacteriology laboratories have a piece of apparatus with an illuminated glass which is lined so that there are many small squares on it. The technician places his petri dish and then counts a representative number of small squares, averages them, and multiples by the total number of squares. Cardboard counting cards may be purchased from biological supply houses or the teacher may wish to set up the overhead projector with an appropriately lined transparency on it for counting purposes.

(c) Routine dilution plating. In the case of professional laboratory work, a culture grown in the above manner may not give accurate enough results because the plates become overgrown with colonies. Thus a method of diluting is routinely used in plating. This method is described below.

Prepare five test tubes, each containing 9 cc of distilled water. Plug the tubes with cotton and sterilize them in a pressure cooker at 15 pounds pressure for 30 minutes. With a sterile pipette, carefully place 1 cc of of the material to be tested in one of the tubes of sterile water. Rotate the tube between the hands rapidly several times to mix thoroughly. Take 1 cc from this test tube and add to a second tube of sterile water, mixing thoroughly. Continue in this manner until all five tubes of sterile water have been inoculated.

This procedure will produce dilutions of the material to be tested of 0.1, 0.01, 0.001, 0.0001, 0.00001. These dilutions are necessary because bacteria may be so concentrated in the sample that it will be impossible to count individual colonies in the next stage of the procedure.

Transfer 1 cc of the liquid in each to the surface of individual sterile petri dishes of nutrient agar. Gently rotate the plate on the table so that the liquid is distributed over the entire surface of the plate. Incubate for 24 hours and count the individual colonies on each plate. On some plates, colonies may be so abundant that it is impossible to count them, while on others there may be very few. Plates having 50 to 100 colonies probably produce the most accurate results. The number of bacteria in the original sample of milk or water can be calculated by multiplying by the proper dilution factor.

In order to plate other milk products, the standard method is to blend them to a liquid form and proceed as detailed above.

C-28. Killing Bacteria With Heat [17, 9, 2, 1]

Prepare 100 ml of nutrient broth and put equal amounts in three test tubes. Plug the tubes with cotton. Allow them to stand several hours before proceeding, so that some of the spores present can become active, reproducing bacteria. Do not let stand long enough for the broth to become cloudy.

Process one of the tubes in a pressure cooker at 15 pounds pressure for 30 minutes. This will kill all organisms, including those in the spore stage, making the contents of the tube sterile.

Put a second tube in a beaker of boiling water for 5 minutes. This will kill bacteria in the vegetative stage, but will not harm the spores that are present.

Incubate all three tubes for 2 or 3 days, examining each daily. It will be expected that the first tube, being sterile, will remain clear. The tube that was in boiling water will become cloudy as the bacteria that were in the spore stage become active and multiply. The tube that was not processed will be cloudier since the original bacteria population included active, vegetative bacteria as well as those in the spore stage.

C-29. Agents for Killing Bacteria [11, 1, 2]

A general method may be used for testing the effectiveness of a number of agents in killing bacteria. This method is described in activity C-20.

(a) Disinfectants and antiseptics. Prepare several petri dishes of seeded agar. With a glass-marking pencil, divide the plates into quarters and number them. Dip blotting paper discs into such antiseptics and disinfectants as iodine, Mercurochrome, Merthiolate, Lysol, and hydrogen peroxide. Alcohol does not produce a good zone of inhibition, probably because of its rapid rate of evaporation. Students are often eager to bring samples of their favorite antiseptics and disinfectants and should be encouraged to do so.

(b) Heavy metals. Many antiseptics and disinfectants are compounds of heavy metals. Familiar examples are Mercurochrome, Metaphen, and Merthiolate, which are compounds of mercury, and silver nitrate and silver lactate, which are compounds of silver.

Silver and copper coins themselves will produce zones of inhibition when placed on seeded agar. Do not put more than two coins on a single plate so that the zones of inhibition will not interfere with each other.

C-30. Determining the Best Method of Washing Dishes

Put some cotton-tipped applicators in a stoppered test tube and sterilize them in an oven at 177°C for 1 hour. Prepare several sterile petri dishes of nutrient agar. Rub one of the cotton-tipped applicators across the back of a dish that has been washed in a dishwashing machine and immediately streak the surface of a petri dish of sterile agar. Streak the surface several times. This will transfer bacteria that may have been on the dish to the agar where they will grow and form colonies.

Do the same with a dish that has been washed and dried by hand. Incubate both plates for 2 or 3 days, examine daily, and count the number of colonies on each. There should be a difference in the colony count. The one with the lower count, of course, represents the better method of washing dishes.

Modifications of this procedure can be used to determine answers to such questions as: with hand-washed dishes, is it better to dry them with a towel or let them air-dry? What is the relative effectiveness of various brands of detergents or soaps?

C-31. Effect of Ultraviolet Light on Microbes [5, 1, 17]

Ultraviolet light of certain wavelengths will kill microorganisms. Bakeries and other food processors expose their products to these wavelengths and greatly reduce their product losses due to mold. Hospitals, factories, and schools are using ultraviolet lamps to disinfect the air.

(a) Molds killed by ultraviolet light. Wet several pieces of bread with water so that they are saturated, and liberally sprinkle dust, which invariably contains spores, on each.

Put one piece of the bread in a moist chamber, such as a mayonnaise jar, and tighten the lid securely. Expose the other pieces to ultraviolet light. Use varying combinations of distance and time for the exposures. Make a record of exposure time and distance and attach it to each jar. Put each piece of bread in a separate moist chamber and tighten each lid securely.

Place the jars containing the bread in a dark place at room temperature and examine daily for a period of 2 or 3 weeks to compare the mold growth in each.

(b) Bacteria killed by ultraviolet light. Prepare nine seeded petri dishes of nutrient agar as described in C-13. Leave the glass covers on three plates, uncover three plates, and cover three others with a plastic wrap. Expose a covered, uncovered, and plastic covered dish to an

ultraviolet lamp, expose three others to an ordinary light bulb, and leave the other three in a dark place. Expose them to each condition for about 15 minutes. If desired, other sets of plates may be prepared and exposed for varying lengths of time. Incubate the plates for 2 or 3 days. Examine them daily for variations in growth rates.

C-32. Evidence of Air Pollutants [8, 5, 1]

Smoke, smog, soot, and dust are suspended particles of solids and/or liquids in the air. The largest of the particles eventually settle out of the air, but some particles are small enough to be kept floating in the atmosphere by the bombardment of air molecules.

These small particles are apt to be of more serious consequence to living things than the larger ones. Since they do not settle out of the atmosphere, they can be carried great distances, may be inhaled by man and other animal life, may be taken into the leaves of green plants, or may act as nuclei for the condensation of other substances in the air.

(a) Very small particles can often be seen in a very bright reflected light even though they are practically invisible in ordinary light. Have pupils observe the motes (small dust particles) in a beam of sunlight as it passes into a darkened corner of the room. Ordinarily one would not be aware of the existence of these small particles.

(b) Place a clean cloth over the intake of a vacuum pump or over the intake hose of a vacuum cleaner. Draw air through the cloth for several minutes.

(c) Remove the rubber bulb from a medicine dropper. Insert a small wad of cotton in the glass tube and replace the bulb. Insert the end of the dropper in a lighted cigarette. Squeeze the bulb a few times to draw the smoke through the cotton. What happens? What conclusions might be drawn as to the nature of tobacco smoke?

(d) Microscope slides smoothly coated with petroleum jelly will collect pollen, dust, and other air pollutants.

(e) Snow can be collected in a plastic sheet, the corners gathered, and the snow allowed to melt. The water may then be placed in a battery jar and allowed to stand until possible pollutants settle out.

(f) Take photographs of various situations in the community which show air being polluted. Three major sources of air pollution involve trash-burning, either at home or in community dumps, burning leaves in the autumn, and industrially produced smokes and gases.

Does your community have other sources of pollution not included in one of these groups? Which ordinances pertaining to air pollution are enforced?

C-33. Effect of Refrigeration on Milk Bacteria [2, 1]

(a) Bacteria counts. Divide a sample of pasteurized milk into two portions. Keep one portion in a refrigerator for 24 hours and the other at room

temperature for the same time. Prepare culture plates of each sample and determine bacterial counts for each as described in C-27.

(b) Growth rate of bacteria in milk. Do a plate count on pasteurized milk directly from a fresh bottle. Record the bacterial count. Keep the bottle in the refrigerator; make counts daily for a week or longer. Graph the results. If the line graph is concave upward, the rate of increase was greater each day.

Do the same with a bottle of milk kept at room temperature. The line graph will be of the same type, but indicates a much more rapid increase each day.